A pharmacogenetic model of alcohol consumption and metabolism. *L. Mustavich[1], P. Miller[1,2], H. Zhao[3,4], K. Kidd[4]* 1) Interdepartmental Program in Computational Biology and Bioinformatics, Yale University; 2) Center for Medical Informatics, Yale University, New Haven, CT 06520; 3) Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, CT 06520; 4) Department of Genetics, Yale University School of Medicine, New Haven, CT 06520.

Several classes of alcohol dehydrogenase and aldehyde dehydrogenase genes, which act in the primary ethanol metabolism pathway, have repeatedly been implicated in alcohol dependence. Certain isoforms are believed to protect against alcohol dependence by causing buildup of the toxic intermediate acetaldehyde, deterring further alcohol consumption. Bitter taste receptor genes also appear to affect ethanol intake, but their combinatorial effect on alcohol dependence, together with the metabolic genes, remains unclear.

We present a compartmental model to explore possible mechanisms underlying the development of alcohol dependence, with the primary aim of determining the extent to which variation in certain genes impacts variation in quantitative traits which may underlie the disease. Previous pharmacokinetic models have studied the time-course of blood ethanol levels, following a single dose of alcohol. We expand upon these models by including acetaldehyde levels, allowing the prediction of further alcohol consumption behavior. Genetics is incorporated into the model through kinetic parameters corresponding to different allelic variants. The result is a theoretical profile for each multi-locus genotype, consisting of a time-course of ethanol and acetaldehyde levels throughout the body, the area under the blood ethanol curve, and other endophenotypes. The model explains known in vivo metabolic measurements and makes testable predictions. Simulations of the current model reveal epistatic interactions among genetic variants of the metabolic and bitter taste receptor genes.

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Variation in the 4q25 Chromosomal Locus Predicts New-onset Atrial Fibrillation after Cardiac Surgery. *S. C. Body[1], C. D. Collard[3], S. K. Shernan[1], A. A. Fox[1], K.-Y. Liu[1], M. D. Ritchie[6], T. E. Perry[1], J. D. Muehlschlegel[1], B. S. Donahue[4], M. Pretorius[4], P. T. Ellinor[8], C. Newton-Cheh[8], C. E. Seidman[2, 7], J. G. Seidman[7], D. S. Herman[7], P. Lichtner[9], T. Meitinger[9], N. J. Brown[5, 6], D. M. Roden[5, 6], D. Darbar[5,6]* 1) Dept Anesthesia, Brigham & Women's Hosp, Boston, MA; 2) Howard Hughes Medical Institute, Brigham and Womens Hospital, Harvard Medical School, Boston, MA; 3) Texas Heart Institute, Saint Lukes Episcopal Hospital, Houston, TX; 4) Department of Anesthesiology, Vanderbilt University School of Medicine, Nashville, TN; 5) Department of Medicine, Vanderbilt University School of Medicine, Nashville, TN; 6) Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN; 7) Department of Genetics, Harvard Medical School, Boston, MA; 8) Cardiovascular Research Center, Massachusetts General Hospital, Boston, MA; 9) Institute of Human Genetics, Helmholtz Zentrum München, Munich, Germany.

Atrial fibrillation (AF) is the most common adverse event following cardiac surgery. We used clinical and genomic data from 3 major cardiovascular surgical programs to determine the role of recently described 4q25 variants in new-onset postoperative AF (PoAF). In a discovery cohort of 566 patients, previously identified 4q25 SNPs were associated with PoAF, accounting for clinical covariates. Haplotype tagging SNPs encompassing ~300kbp of 4q25 were genotyped in a validation cohort of 940 patients. Multivariable logistic model of the occurrence of postoperative AF confirmed older age and prior AF to be risk factors for the development of PoAF. 4q25 SNPs previously associated with ambulatory AF, and other SNPs in linkage disequilibrium with those, described a haplotype that was significantly associated with new-onset PoAF, accounting for family-wise error. Odds ratio for the associated SNPs ranged between 1.61 and 2.26 ($P<10-6$) in the validation cohort, after accounting for clinical covariates. rs2200733 and rs2220427 were the most highly associated SNPs. We have shown in discovery and validation cohorts that non-coding 4q25 SNPs associated with ambulatory AF are also strongly associated with PoAF.

Clinical evaluation of a custom genome-wide microarray for the copy number changes in hematologic neoplasmas. *L. Shao, S. Kang, S. W. Cheung, A. Patel* Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX.

Conventional chromosome analysis in leukemic cells is of intrinsically low resolution. Emerging evidence suggests that chromosomal copy number changes are important predictors of disease progression and prognosis in hematological diseases. The most recent innovation in whole genome analysis, namely aCGH, offers a high resolution view of the leukemic cell genome. We present a custom genome-wide oligonucleotide array which is also targeted for genes involved in carcinogenesis for use in all hematological disorders characterized by copy number changes. We analyzed 21 patients with different hematologic neoplasms using this chip. The indications included chronic lymphocytic leukemia (N=11), myelodysplasia (N=3), acute myeloid leukemia (N=2), multiple myeloma (N=2), amyloidosis, eosinophila, and Burkitt lymphoma. All patients had concurrent karyotype and/or FISH analysis. At least one copy number change was detected in 10 out of 21 patients. Four of them were consistent with chromosome and/or FISH analysis while 6 of them had additional findings of cryptic deletions or duplications. The cryptic findings included a loss of 0.8 Mb in 1q25 including the ABL2 oncogene, a gain of 5 Mb in 2p16 including REL and BCL11A oncogenes, a loss of 0.9 Mb in 8q24 including the metastasis suppressing gene MTSS1, a loss of 5.5 Mb in 9p21 including the CDKN2A tumor suppressor gene, a loss of 2.5 Mb in 11q25, and a loss of 2.1 Mb in 17q12 including the NF1 gene. In addition, partial trisomies of chromosome 2p, 4q, 11q, and 15q, and der(8) were also observed. In a patient with a marker chromosome, aCGH revealed the marker to be of chromosome 7 origin. As expected, an apparently balanced inversion on 3q was not detected by aCGH. In total, 60% of patients with abnormal chromosome and/or FISH analysis displayed at least one additional cryptic change by aCGH. Our results suggest that a subset of potentially significant genomic alterations may be missed by the current available cytogenetic techniques. Furthermore, this pilot study clearly shows high sensitivity and specificity for whole genome microarray analysis for potential use in routine screening in hematological neoplasms.

Data coordinating infrastructure for the Autism Genome Project. *O. Stein, Autism Genome Project* Battelle Center for Mathematical Medicine, The Research Institute at Nationwide Children's Hospital, Columbus, OH.

The Autism Genome Project is an international collaboration dedicated to gene discovery in Autism (AD). Due to the large quantities of clinical and molecular data, and the distribution of the project across many clinical groups and laboratories, the AGP has established a Data Coordinating Center (DCC) which houses a state-of-the-art infrastructure for data input, data cleaning and curation, data output, and large-scale data archiving. The core DCC infrastructure includes two 64 bit MySQL Linux database servers, and a webserver (LAMP) which is used by collaborators for uploads/downloads. The servers are configured as master and slave, which sit in different locations as a disaster recovery precaution: the master acts as the sole data input entity, and is automatically replicated to the slave. Both master and slave can be used to retrieve data, balancing the load each server has to carry; administrative tasks such as backups are also done on the slave. The system capacity can be extended by additional cloning of slave machines. The main interface for data submission is the web application, which automatically vets item-level clinical data (ADI, ADOS, etc), uploaded over a secure channel in simple csv (comma separated value) format, upon input, checking for illegal variable values and logical errors (eg age of onset prior to current age) with immediate feedback given to user if problems are detected. Molecular data ranging from 10k to 1M SNP chip data are imported and cleaned through a semi-automated process, in which files are output from the database, run through error-detection programs that write output directly in SQL command format for execution in the database, with iterative processing until all errors are removed from output files. AGP participants can download raw and cleaned data via the same web server. All raw data, including images from the large SNP experiments, are stored either within the database or (in the case of images) in an automated terabyte tape storage facility. The current footprint of the database is ~400GB and ~10 TB raw images on tape. This includes over 19000 samples, over 1M phenotypic datapoints, and ~2.5B genotypes.

Correlation of *HMGA2* gene variation with height in specific pediatric age categories. *J. P. Bradfield[1], M. Li[2], C. E. Kim[1], K. Annaiah[1], E. Santa[1], J. T. Glessner[1], E. C. Frackelton[1], F. G. Otieno[1], J. L. Shaner[1], R. M. Smith[1], A. W. Eckert[1], M. Imielinski[1], R. M. Chiavacci[1], R. L. Berkowitz[3], H. Hakonarson[1,4,5], S. F. A. Grant[1,4,5]* 1) Center for Applied Genomics, Abramson Research Center, Children's Hospital of Philadelphia, Philadelphia, PA; 2) Department of Biostatistics and Epidemiology, University of Pennsylvania, Philadelphia, PA; 3) Weight and Eating Disorders Program, Department of Psychiatry, University of Pennsylvania School of Medicine, 3535 Market Street, Philadelphia, PA; 4) Division of Human Genetics, Abramson Research Center, Children's Hospital of Philadelphia, Philadelphia, PA; 5) Department of Pediatrics, University of Pennsylvania School of Medicine Philadelphia, PA.

Recently an association was demonstrated between the single nucleotide polymorphism (SNP), rs1042725, within the *HMGA2* locus and height as a consequence of a genome wide association (GWA) study of this trait in adults; this observation was also reported in children aged 7-11 years old. We examined in our Caucasian childhood cohort the effects of two strong surrogates for this SNP at this locus with height, rs8756 and rs7968902, with respect to the same pediatric age category but also in children grouped separately as younger and older. Utilizing data from an ongoing GWA study in our cohort of 2,619 Caucasian children with measurements for height, we investigated the association of the previously reported variation at the *HMGA2* locus with this height treated as a quantitative trait (age and sex corrected) in childhood in the 2-6 (n=706), 7-11 (n=617) and 12-18 (n=1293) years old categories. The minor alleles of rs8756 and rs7968902 respectively (strong surrogates for rs1042725 i.e. r^2= 0.873 and 0.761 in the CEU HapMap respectively) were significantly correlated with height in the 7-11 years old age group (P = 3.53×10^{-3} and 2.82×10^{-4}, respectively). However in the 2-6 and 12-18 years old age groups, no correlation was observed. In summary, we observe a strong correlation with height in same age group of 7-11 years old as has been previously reported. However, in the under 7s and the over 11s, no such association was observed.

Follow up analysis of genome-wide association data and replication identifies novel loci for type 1 diabetes. *S. F. A. Grant[1,2,3], H. Q. Qu[4], J. P. Bradfield[1], L. Marchand[4], M. Imielinski[1], C. E. Kim[1], J. T. Glessner[1], R. Grabs[4], S. P. Taback[5], E. C. Frackelton[1], K. Annaiah[1], M. L. Lawson[6], R. W. Grundmeier[7,8], C. A. Stanley[9], S. E. Kirsch[10], D. Waggott[11], A. D. Paterson[12], D. S. Monos[3,13], C. Polychronakos[4], H. Hakonarson[1,2,3]* 1) Center for Applied Genomics, Abramson Research Center, Children's Hospital of Philadelphia (CHOP), PA; 2) Division of Human Genetics, Abramson Research Center, CHOP, PA; 3) Dept of Pediatrics, University of Pennsylvania School of Medicine, Philadelphia, PA; 4) Depts of Pediatrics and Human Genetics, McGill University, Montreal, Canada; 5) Dept Pediatrics and Child Health, University of Manitoba, Winnipeg, Canada; 6) Division of Endocrinology, Childrens Hospital of Eastern Ontario, University of Ottawa, Canada; 7) Pediatric Research Consortium, CHOP, PA; 8) Dept of Biostatistics and Epidemiology, University of Pennsylvania School of Medicine, PA; 9) Division of Endocrinology, CHOP, PA; 10) Markham-Stouffville Hospital, Markham, Canada; 11) Samuel Lunenfeld Research Institute of Mount Sinai Hospital, Toronto, Canada; 12) Dept of Public Health Sciences, Hospital for Sick Kids, Toronto, Canada; 13) Dept of Pathology and Laboratory Medicine, Abramson Research Center, CHOP, PA.

Two recent genome wide association (GWA) studies have revealed novel loci for type 1 diabetes (T1D). To fully utilize the GWA data we had obtained by genotyping 563 T1D probands and 1,146 controls, as well as 483 case-parent trios, using the Illumina HH550 BeadChip, we selected 982 markers with $P<0.05$ in both GWA cohorts. Genotyping these in an independent set of 636 nuclear families with 974 affected offspring revealed 75 markers that also had $P<0.05$ in this third cohort. Among these, 6 SNPs in 5 novel loci also had P<0.05 in the WTCCC dataset and were further tested in 1,303 T1D probands from DCCT/EDIC plus 1,673 controls. Two markers (rs9976767 and rs3757247) remained significant after adjusting for the number of tests in this last cohort; they reside in *UBASH3A* (OR=1.16; combined $P=2.33\text{x}10^{-8}$) and *BACH2* (OR=1.13; combined $P=1.25\text{x}10^{-6}$). The two genes are both biologically relevant to autoimmunity.

Genetic Variation in Late Preterm and Term Infants with Respiratory Distress. *K. Borowski[1], J. M. Dagle[2], J. C. Murray[2]* 1) Dept OB & GYN, Univ Iowa, Iowa City, IA; 2) Dept Pediatrics, Univ Iowa, Iowa City, IA.

BACKGROUND AND OBJECTIVE: Respiratory distress is a common morbidity in the late preterm infant. Single gene mutations in *SFTPB* and *ABCA3* as well as an increased risk for siblings suggest a genetic component to respiratory distress. The objective of this study is to evaluate genetic variants in fetal and maternal steroid metabolism genes and key pulmonary function genes in cases of late preterm and term respiratory distress. DESIGN: Utilizing a candidate gene approach, we analyzed 43 tagged SNPs in 15 genes within the following categories: glucocorticoid metabolism pathway, electrolyte/fluid balance, surfactant proteins and nitric oxide. Inclusion criteria included: respiratory distress with at least 24 hours of oxygen requirement, respiratory symptoms, abnormal xray findings and gestational age 33-41 weeks. Exclusion criteria included: multiple congenital anomalies, meconium aspiration syndrome, culture proven sepsis and surgery requiring ventilation. DNA was isolated from blood leukocytes and saliva/buccal swabs using Qiagen kits. Allelic discrimination was then performed using TaqMan genotyping assays from ABI. Analysis was performed using family based analysis/tranmission disequilibrium test (TDT). RESULTS: 92 probands and their families were included in the analysis. 23 probands met more stringent requirements with surfactant administration. Replication was then performed in 34 families. In the initial analysis four genes suggested association: *CRHR1* p=0.029, *SFTPB* p=0.013, *SCNN1A* p=0.035 and *SCNN1G* p=0.015. These did not meet a Bonferroni correction significance of p=0.0011. In replication *SCNN1A* and *CRHR1* had a p value of 0.02. A haplotype analysis of *SCNN1A*, *SCNN1B* and *SCNN1G* suggested a significant overtransmission with a Z score of 3.39 and p value of 0.0007. CONCLUSION: There is a suggestive association with SCNN1A and CRHR1 and respiratory distress in the late preterm and term neonate. These genes may play modifier roles in the development of respiratory distress in the late preterm and term infant. Furthermore, the SCNN1 gene family may act together to influence respiratory distress in the neonate.

Genetic Effects in the Leukotriene Biosynthesis Pathway and Association with Atherosclerosis. *D. Crosslin[1], S. Shah[1,2], S. Nelson[1], C. Haynes[1], J. Connelly[1], S. Gadson[1], P. Goldschmidt-Clermont[3], J. Vance[4], C. Granger[2], D. Seo[3], S. Gregory[1], W. Kraus[2], E. Hauser[1]* 1) Center for Human Genetics, Duke University Medical Center, Durham, NC; 2) Division of Cardiovascular Medicine, Duke University Medical Center, Durham, NC; 3) Miller School of Medicine, University of Miami, Miami, FL; 4) Institute of Human Genomics, University of Miami, Miami, FL.

To understand the role of the leukotriene (LKT) pathway in atherosclerosis pathophysiology, we analyzed genotype, expression and clinical data from 78 human aortas to evaluate correlations in the LKT biosynthetic cascade. We also evaluated the association between cardiovascular disease (CVD) phenotypes, expression and previously reported *ALOX5AP* and *LTA4* haplotypes. We analyzed SNPs found in HapA, HapB (*ALOX5AP*) and HapK (*LTA4H*) as well as SNPs found in the *ALOX5* gene. We also used Gene Set Enrichment Analysis (GSEA) to test correlation between expression and the phenotypes raised lesion mapping (RL) and Sudan IV staining (S4). Our results suggest the importance of using pathway-based modeling for evaluating the genomics of atherosclerosis susceptibility. All three genes had at least one SNP with a *cis* and *trans* effect and all effects for a given gene had a single target. Our results suggest that HapA and the relationship to expression levels for *ALOX5AP* ($p = 0.03$) and *ALOX5* ($p = 0.06$) may be an important feature that links the genetic and genomic results. Neither HapK nor HapB were associated with any of the expression values. For GSEA, the custom LKT biosynthesis gene set for RL had an enrichment score (ES) of 0.6897 ($p = 0.004$) and the S4 produced an ES of 0.6349 ($p = 0.009$). Our results support previous association studies; however, the relationship between genetic variation and CVD outcomes is not driven by a single gene or SNP. By exploring the pathway in terms of risk for atherosclerosis, coronary artery disease and myocardial infarction along with genotypic effects on expression, rather than the looking at each component in isolation, we observe significant complexity in the interactions that may alter risk profiles related to genetic variation.

GWAS identifies genes affecting quantitative variation of white blood cell count in African-American children on 1q23. *M. Imielinski[1,2], J. P. Bradfield[1], K. Annaiah[1], C. E. Kim[1], A. W. Eckert[1], J. T. Glessner[1], K. Thomas[1], G. Otieno[1], E. Santa[1], E. Frackleton[1], R. M. Chiavacci[1], S. F. A. Grant[1,3,4], H. Hakonarson[1,3,4]* 1) Center for Applied Genomics, Children's Hospital of Philadelphia, Philadelphia, PA, 19104, USA; 2) Department of Pathology, Massachusetts General Hospital, Boston, MA. 02114; 3) Department of Pediatrics and Division of Human Genetics, The Children's Hospital of Philadelphia, Philadelphia, PA, 19104, USA; 4) Department of Pediatrics, University of Pennsylvania School of Medicine, Philadelphia, PA, 19104, USA;.

White blood cells (WBC) mediate immunity and inflammation in normal healthy individuals. WBC count elevation has also been identified as an independent risk factor for acute myocardial infarction, cerebrovascular accident, and all-cause mortality. A recent admixture mapping study found that the Duffy antigen locus on 1q23 associated with WBC count in African American adults. To further gain insight into the genetic basis of WBC regulation in health and disease, we conducted a genome-wide association study of WBC count in a cohort of 2275 Caucasian and 2734 African-American children genotyped on Illumina 550K SNP arrays. In the African-American cohort, we found normal variation in WBC count and absolute neutrophil count associated to a genome-wide significant level (<9E-8) with 65 SNPs spanning an 8 MB region on chromosome 1q23. This association replicated robustly in an independent cohort of 500 African American children. No similar or genome-wide significant associated signal was found in the Caucasian cohort. The region of association includes the Duffy antigen locus, as well as numerous genes involved in WBC development and inflammation. These results point to novel mechanisms underlying the regulation of hematopoiesis in healthy African American individuals. They also support a link between the selective pressure imposed by the malaria parasite Plasmodium vivax and immune system evolution. Further investigation is warranted to determine whether these genes confer clinically significant risk or protection to disease in African Americans.

Association of the *BANK1* R61H variant with systemic lupus erythematosus in Americans of European and African ancestry. *K. Sullivan[1,2], S. F. A. Grant[1,3,4], M. A. Petri[5], J. P. Bradfield[3], C. E. Kim[3], E. Santa[3], K. Annaiah[3], E. C. Frackelton[3], J. T. Glessner[3], F. G. Otieno[3], J. L. Shaner[3], R. M. Smith[3], A. W. Eckert[3], R. M. Chiavacci[3], M. Imielinski[3], H. Hakonarson[1,3,4]* 1) Department of Pediatrics, University of Pennsylvania School of Medicine, Philadelphia, PA; 2) Division of Allergy and Immunology, Abramson Research Center, Children's Hospital of Philadelphia, Philadelphia,; 3) Center for Applied Genomics, Abramson Research Center, Children's Hospital of Philadelphia, Philadelphia, PA; 4) Division of Human Genetics, Abramson Research Center, Children's Hospital of Philadelphia, Philadelphia, PA; 5) Division of Rheumatology, Johns Hopkins School of Medicine, Baltimore, MD.

Recently an association was demonstrated between a common coding variant, rs10516487 (R61H), within the B-cell gene *BANK1* and systemic lupus erythematosus (SLE) as a consequence of a genome wide association (GWA) study of this disease in European and Argentinean populations. In a bid for replication, we examined the effects of the R61H variant with respect to SLE in our genotyped American cohorts of European and African ancestry. Utilizing data from our ongoing GWA study in our cohort of 178 Caucasian SLE cases and 1808 Caucasian population-based controls plus 148 African American (AA) SLE cases and 1894 AA population-based controls we investigated the association of the previously non-synonymous SNP at the *BANK1* locus with the disease in the two ethnicities separately. The minor allele frequency (MAF) of rs10516487 in the Caucasian cases was 22.6% while it was 31.2% in Caucasian controls, yielding a protective odds ratio (OR) of 0.64 (95% CI 0.49-0.85; one-sided $P=7.07 \times 10^{-4}$). Furthermore, the MAF of rs10516487 in the AA cases was 18.7% while it was 23.3% in AA controls, yielding a protective OR of 0.75 (95% CI 0.55-1.034; one-sided *P*=0.039). The OR of the *BANK1* variant in our study cohorts is highly comparable with that reported previously in a South American/European SLE case-control cohort (OR=0.72). As such, R61H in the *BANK1* gene confers a similar magnitude of SLE protection, not only in European Americans, but also in African Americans.

Genome Wide Association of Copy Number Variations (CNVs) in Type 1 Diabetes (T1D) Identifies Novel Genes in Previously Associated T1D Pathways. *H. Hakonarson[1], J. T. Glessner[1], H. Q. Qu[2], K. Wang[1], J. P. Bradfield[1], C. E. Kim[1], L. Marchand[2], S. F. A. Grant[1], C. Polychronakos[2]* 1) Center for Applied Genomics, Children's Hospital of Philadelphia, Philadelphia, PA; 2) Departments of Pediatrics and Human Genetics, McGill University, Montreal H3H 1P3, Que'bec, Canada.

T1D is an autoimmune disease with permanent destruction of the insulin producing beta cells. To determine if CNVs contribute to T1D, we performed extensive Quality Control (QC) measures on our Illumina550 GWAS data, including call rate > 98%, SD of normalized intensity (LRR) < 0.35, low wave artifact correlating with GC content due to hybridization bias of low full length DNA quant -0.2X0.4, and proper balance of B-Allele Frequency (BAF). The samples that passed QC for association included 504 Caucasian children with T1D including 292 complete trios in comparison with 3979 controls. Key performance features of the Illumina array for CNV include random placement of SNP specific beads on each array, 18 fold assay redundancy, and confirmatory expected genotype color contrast to supplement intensity data. PennCNV (Wang et al, 2007) was used to call CNVs applying a Hidden Markov Model and Viterbi Algorithm. Observance of CNV at each SNP was evaluated genome wide with chi square significance testing. Here we report statistical local minimums in reference to a region of nominal significance including SNPs residing within 1MB. After review, 47 CNV regions (29 resided on genes) with at least 2 cases each were associated with T1D. Genes with direct functional relevance to T1D impacted by CNVs included IGFBP4, SORBS1, SCG, NGFR, PTPRT, MAML2, CCND1, BCMO1, EPX, and KCNK2. Functional clustering of independently associated results using Fisher's combined probability test provided: Immune system (3 loci, 7 CNVs p= 1.9E-4), Insulin (2 loci, 4 CNVs p= 1.6E-3), Pancreas or liver expression (4 loci, 8 CNVs p= 2.3E-3), CNS development (5 loci,16 CNVs, p= 1.0E-6), Vitamin A (1 locus, 3 CNVs 5.2E-3), Cell cycle signaling (10 loci, 29 CNVs p= 8.0E-7), and Membrane channels (4 loci,13 CNVs p= 1.6E-5). We conclude that 29 genes harboring 80 CNVs in previously associated T1D pathways affecting 70 T1D cases account for up to 14% of cases.

Genome Wide Association of Copy Number Variations (CNVs) in Schizophrenia Identifies Novel Genes in Glutamate Receptor Networks. *J. T. Glessner[1], K. Wang[1], O. Krastoshevsky[2], J. P. Bradfield[1], C. E. Kim[1], S. Shin[3], N. R. Mendell[3], S. F. A. Grant[1], J. Sebat[4], D. L. Levy[2], H. Hakonarson[1]* 1) Center for Applied Genomics, The Childrens Hospital of Philadelphia, Philadelphia, PA; 2) Psychology Research Laboratory, McLean Hospital, Belmont, MA; 3) Department of Applied Mathematics and Statistics, State University of New York at Stony Brook, Stony Brook, NY; 4) Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Schizophrenia is a late adolescent-onset psychiatric disease typically characterized by delusions, hallucinations and thought disturbances. We have confirmed association of DISC1, GRIA4, and CHN2 with schizophrenia. To determine if CNVs contribute to the development of schizophrenia, we performed extensive QC on Illumina550 data including call rate > 98%, SD of normalized intensity (LRR) < 0.35, low wave artifact correlating with GC content due to hybridization bias of low full length DNA quant -0.2X0.4, and proper balance of B-Allele Frequency (BAF). Following QC, 136 Caucasian individuals with schizophrenia including 36 trios were analyzed with 1,338 controls. Key Illumina array features for CNV include random placement of SNP specific beads on each array, 18 fold assay redundancy, and expected genotype color contrast to supplement intensity data. PennCNV (Wang et al, 2007) was used to call CNVs applying a Hidden Markov Model. CNV at each SNP was evaluated genome wide with chi square testing. Statistical local minimums were reported in reference to a region of nominal significance of SNPs residing within 1MB. Associated regions were reviewed for call accuracy, lack of peninsulas created by boundary truncation, continuity of coverage, and compared with the Database for Genomic Variants. After review, 11 CNV regions (7 resided on genes) remained with at least 2 CNV cases. Genes with functional relevance to schizophrenia included NTS, GRIK5, and GRM5 (All p=8.5E-3). Functional clustering of independently associated results provided: ionotropic glutamate receptor activity (p= 5.8E-4 GRIK1, GRIA4, GRIN3A, and GRIK5). We conclude that 6 genes harboring 9 CNVs (in 9 cases) in neurotransmission may account for a significant number of schizophrenia cases.

A Genome Wide Association Study Identifies Novel Inflammatory Bowel Disease Susceptibility Loci on 20q13 and 21q22 in Patients with Pediatric Onset IBD. *R. N. Baldassano[1,2], S. Kugathasan[3], J. P. Bradfield[4], P. M. A. Sleiman[4], M. Imielinski[4], S. L. Guthery[5], S. Cucchiara[6], T. Willson[7], E. Bonkowski[7], N. Peterson[3], D. J. Abrams[2], R. Grundmeier[4], P. Mamula[3], G. Tomer[7], D. A. Piccoli[2], D. S. Monos[8], V. Annese[9], L. A. Denson[7], S. F. A. Grant[1,4,10], H. Hakonarson[1,4,10]* 1) Department of Pediatrics, University of Pennsylvania School of Medicine, Philadelphia, PA; 2) Division of Gastroenterology, Childrens Hospital of Philadelphia (CHOP), Philadelphia, PA; 3) Department of Pediatrics, Childrens Research Institute & Medical College of Wisconsin, Milwaukee, WI; 4) Center for Applied Genomics, Abramson Research Center, CHOP, PA; 5) Department of Pediatrics, University of Utah School of Medicine and Primary Childrens Medical Center, Salt Lake City, UT; 6) Pediatric Gastroenterology & Liver Unit, Sapienza University of Rome, Italy; 7) Division of Gastroenterology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH; 8) Divisions of Immunology and Pathology, CHOP, Philadelphia, PA; 9) Units of Gastroenterology & Endoscopy, IRCCS, Casa Sollievo della Sofferenza Hospital, San Giovanni Rotondo, Italy; 10) Division of Human Genetics, Abramson Research Center, CHOP, PA.

Inflammatory bowel disease (IBD) is a common inflammatory disorder with complex etiology. It is characterized by two distinct phenotypes: Crohns disease (CD) and ulcerative colitis (UC). Previously reported GWA studies have identified genetic variation accounting for a small portion of the overall genetic susceptibility to CD and an even smaller contribution to UC pathogenesis. We hypothesized that stratification of IBD by age of onset may identify new IBD genes. To that end, we performed a GWA analysis in a cohort of 1,011 pediatric onset IBD cases and 4,250 matched controls. We identified, and replicated, significantly associated novel loci on chromosomes 20q13 (rs2315008 allele T and rs4809330 allele A; $P = 6.30 \times 10^{-8}$ and 6.95×10^{-8}, respectively; odds ratio (OR) = 0.74 for both) and 21q22 (rs2836878 allele A; $P = 6.01 \times 10^{-8}$; OR = 0.73) located close to the *TNFRSF6B* and *PSMG1* genes, respectively.

ORMDL3 variants associated with asthma susceptibility in North Americans of European ancestry. *K. Annaiah[1], P. M. A. Sleiman[1], M. Imielinski[1], J. P. Bradfield[1], C. E. Kim[1], E. C. Frackelton[1], J. T. Glessner[1], F. G. Otieno[1], E. Santa[1], W. Glaberson[1], M. Garris[1], R. Chiavacci[1], J. Allen[2], J. Spergel[3], R. Grundmeier[4], M. M. Grunstein[2], M. Magnusson[5], H. Bisgaard[6], S. F. A. Grant[1,5], H. Hakonarson[1,2,5]* 1) Center for Applied Genomics, The Childrens Hospital of Philadelphia; 2) Division of Pulmonary Medicine, The Childrens Hospital of Philadelphia; 3) Division of Allergy and Immunology, The Childrens Hospital of Philadelphia; 4) Department of Bioinformatics, The Childrens Hospital of Philadelphia; 5) Department of Pediatrics, The Childrens Hospital of Philadelphia; 6) Department of Health Sciences, University of Copenhagen.

Asthma is the most common chronic disease in children across all developed countries. The first GWA study of asthma predisposition was recently published by Moffatt et al. In that study 317,000 SNPs were typed in 994 patients with childhood-onset asthma, resulting in the identification of a novel locus on chromosome 17q12 containing multiple genes and associated markers. To determine if ORMDL3 is a genetic risk factor for asthma in North American Caucasians and African Americans we sought to replicate the association with the 10 most significantly associated SNPs in the study by Moffatt et al, in a large pediatric asthma cohort collected at the Childrens Hospital of Philadelphia. The study included 3390 North Americans of European ancestry; 807 patients with physician-diagnosed asthma and 2583 disease-free controls and 3429 African American samples, 1456 asthma patients and 1973 controls. High throughput genome wide SNP genotyping was carried out at the center for applied genomics on the HumanHap550 BeadChip. This study has replicated the reported association between asthma and variants in and around ORMDL3 in a cohort of North American Caucasian asthmatics. Seven of the nine SNPs that were tested showed significant association (P-value range 0.004-0.037 OR range 1.1-0.84). However, the odds ratios obtained in our cohort are significantly lower than those reported by Moffatt et al., which may be a case of the winners curse. In contrast, no association was detected between these markers and asthma in African-Americans.

Genome-wide analysis of structural variation by pair-end mapping. *V. Kumar[1], J. H. H. Tan[1], Y. Zhu[1], F. Yao[2], Y. Ruan[2], M. Seielstad[1]* 1) HUMAN GENETICS, GENOME INSTITUTE OF SINGAPORE, SINGAPORE; 2) GENOME TECHNOLOGY AND BIOLOGY, GENOME INSTITUTE OF SINGAPORE, SINGAPORE.

Structural variation (SV) can be defined as all genomic changes that are not single base-pair substitutions. Such variation includes insertions, deletions, inversions, duplications and translocations of DNA sequences, and encompasses copy-number differences. A number of studies have shown that SV is highly variable within the normal human population. Systematic analysis of these variations will provide fundamental knowledge to understand the normal human genetic diversity and to distinguish from pathogenic alterations. However, identifying them still remains a technical challenge. We use high-throughput and massive paired-end mapping (PEM), to identify SVs. PEM involves shearing and purifying of intact genomic DNA to yield DNA fragment of ~10 kb. Following this, the DNA fragments were methylated with EcoP15I, circularized, digested with EcoP15I and subsequently fragments containing the paired ends were isolated by streptavidin-affinity purifications. This was followed by massive parallel sequencing and mapping of paired ends to identify SVs, both, reported and novel. Since the fragment size was ~10 kb, it also enabled us to identify fine-scale SVs along with the precise mapping of the break points.

Developmental delay and late onset myoclonus in a patient with interstitial deletion of chr 6q21q22.31. *E. Andermann[1,2,3], J. Lavoie[4,5], F. Andermann[2,6]* 1) Neurogenetics Unit, Montreal Neurological Hospital and Institute, Montreal, Quebec, Canada; 2) Department of Neurology and Neurosurgery, McGill University, Montreal, Quebec, Canada; 3) Department of Human Genetics, McGill University, Montreal, Quebec, Canada; 4) Department of Pathology, Montreal Children's Hospital, Montreal, Quebec, Canada; 5) Department of Pathology, McGill University, Montreal, Quebec, Canada; 6) Department of Paediatrics, McGill University, Montreal, Quebec, Canada.

Rationale: Chromosomal abnormalities are often associated with mental retardation, dysmorphic features and seizures. Interstitial deletions of chr 6q are relatively rare and usually not associated with epilepsy. Methods: A 30-year-old patient had a history of a single febrile seizure at the age of 8 months, mild to moderate developmental delay, and dysmorphic features. In his late twenties, he developed myoclonic jerks. Detailed examination and family history was performed. The patient had prolonged day and night telemetry recording, as well as MRI studies. Karyotype was performed, as well as FISH studies and array CGH. Results: On examination, the patient has mild dysmetria and frequent myoclonic jerks involving the upper extremities. There is no evidence of recent cognitive deterioration. Prolonged video-telemetry revealed extremely frequent myoclonic jerks arising from the right or left upper extremity. No epileptiform abnormality was noted during the jerks or interictally. MRI showed mild cerebellar vermian atrophy or hypoplasia. Karyotype revealed a subtle interstitial deletion of chr 6q. Array CGH employing an oligonucleotide array confirmed a deletion of chr 6q at 6q21-6q22.31. The extent of the deletion is estimated to be 7.6 Mb. Conclusions: Although deletions of chr 6q are usually not associated with epilepsy, the exact deletion described in our patient has not been reported previously, to our knowledge. Phenotypic variation is in large part due to differences in size and location of the segmental aneuploidy. The patient does not have evidence for progressive myoclonus epilepsy, although Kufs disease cannot be entirely ruled out.

13q14 Tumor Suppressor Candidate KCNRG Promotes Apoptosis and Arrests Division of the Tumor Cell Lines.

A. Birerdinc[1], E. Nohelty[1], J. Baughman[1], G. Manyam[1], A. Marakhonov[2], E. Nikitin[2,3], M. Skoblov[2], V. Chandhoke[1], A. Baranova[1,2] 1) Molecular and Microbiology, George Mason University, Fairfax, VA; 2) Research Center for Medical Genetics, RAMS, Moskvorechie Str., 1, Moscow, Russian Federation; 3) Hematology Research Center of Russia , Moscow , Russian Federation.

Deletions of 13q14 have been shown to play a role in the development of chronic lymphocytic leukemia (CLL) and the progression of multiple myeloma (MM). We created a detailed map of transcripts located in 13q14 area and pinpointed the KCNRG gene encoding a potassium channel inhibitor protein as a primary candidate for MM/CLL TSG. KCNRG encodes a protein with a strong homology to the tetramerization domain of voltage-gated K+ channels. This protein may interfere with the normal assembly of the K+ channel proteins, thereby suppressing the potassium currents. Experiments producing stable overexpression of KCNRG-L and KCNRG-S protein isoforms in the model cell lines LNCaP, HL60 and RPMI8226 demonstrated significant suppression of proliferation rates. Both stably and transiently KCNRG-transfected LNCaP and RPMI8226 cells demonstrated drastic phenotype changes, notably the presence of cells with lobular nuclei. In LnCaP cells, an isoform KCNRG-L has been shown to influence the processes of cell adhesion and contact inhibition. Caspase-Glo apoptosis assays showed a significant 26% increase in apoptosis for transfected cells of RPMI8226 line. Chemiluminescent apoptosis and proliferation assays were independently confirmed by FACS analysis using PI and annexin V. RealTime PCR analysis revealed that KCNRG mRNA levels are significantly decreased ($P < 0.02$) in the excised lymphoid tissue of the diffuse large B-cell lymphoma patients (DLBL, $N = 11$) as compared to the lymphoid samples of normal controls ($N = 6$). In stage I and II B-cell lymphoma samples ($N= 34$) KCNRG mRNA levels were higher compared to stage IV lymphomas ($N=4$). These data point to KCNRG as a strong candidate TSG for CLL, MM and other human tumors with 13q14 rearrangements. Supported by NIH R1R15CA113331-01 and Russian Fund for Basic Research RFFI No 07-04-12232 (ofi-a).

Small molecular inhibitors of the potassium channels as potential therapeutic agents for chronic lymphocytic leukemia. *A. Baranova[1,2], M. Skoblov[2], A. Marakhonov[2], B. Biderman[3], A. Birerdinc[2], V. Chandhoke[1], E. Nikitin[3], A. Sudarikov[3]* 1) Molecular and Microbiology, George Mason University, Fairfax, VA; 2) Research Center for Medical Genetics RAMS, Moscow, Russian Federation; 3) Hematology Research Center of Russia, Moscow, Russian Federation.

Region q14 of human chromosome 13 harbors a critical tumor suppressor gene (TSG) for Chronic Lymphocytic Leukemia and some other malignancies. Recently, we described CLL gene candidate KCNRG (K+ Channel Negatively Regulating Gene). KCNRG is significantly similar to the tetramerization domain of voltage-gated K+ channels (Kv channels) and is capable of the suppression of Kv currents. Studies of the proliferation and apoptosis of the cell lines strongly suggest that KCNRG may act as a tumor suppressor gene for the development or progression of various types of cancers, including CLL. If KCNRG indeed plays a role in CLL tumorigenesis, it might define a novel class of human tumor suppressor genes with a mechanism of action that can be relatively easily reproduced by pharmacological means. We hypothesized that K+ channel inhibitors could be used to make up for a loss of KCNRG activity in CLL. The excellent records for nanomolar concentrations of K+ channel blockers used in the management of epilepsy, stroke, and cardiac arrhythmias enhances the attractiveness of this therapeutic approach. Therefore, we started an initial evaluation of the known non-protein K+ channel blockers as apoptotic inducers in primary CLL cells using Chemiluminescent CaspaseGlo assays. For 9 out of 24 tested K+ channel inhibitors, including anti-hypertensive agent verapamil, CLL specific cytotoxic action has been demonstrated. Additionally, it has been noted that one of the control compounds, linoleic acid, possesses specific activities extending survival of the primary CLL cells in vitro. Supported by NIH R1R15CA113331-01 KCNRG gene as candidate tumor suppressor for CLL and MM (2005-2009) and Russian Fund for Basic Research RFFI No 07-04-12232 (ofi-a).

Novel *GNE* mutations in patients of non-Middle Eastern descent with autosomal recessive hereditary inclusion body myopathy. *C. Saechao[1], Y. Valles-Ayoub[1], A. Haghighatgoo[1], S. Esfandiarifard[1], C. Riley[1], M. Pietruszka[1], D. Darvish[1,2]* 1) HIBM Research Group, Encino, Ca; 2) VA Greater Los Angeles (VA-GLA/UCLA), Los Angeles, CA.

The most prevalent form of autosomal recessive (AR) hereditary inclusion-body myopathy (HIBM) is originally described in Iranian-Jewish families. AR HIBM is a progressive muscle wasting disease characterized by early adult onset degeneration of proximal and distal muscles, often sparing the quadriceps. Mutations in the UDP- N-acetylglucosamine 2-epimerase/ N-acetylmannosamine kinase gene (*GNE*) on chromosome 9p12-13 are associated with AR HIBM. In the present study, we have identified the nucleotide sequence of the *GNE* coding region for twenty patients, and noted nine novel mutations. Of these twenty patients, one is homozygous for the M712T founder Middle-Eastern mutation, and one is homozygous for the V572L founder Asian mutation. Thirteen patients, eight of whom are compound heterozygotes, carry the novel mutations. These *GNE* mutations should be considered in clinical and research testing protocols along with previously reported mutations related to HIBM.

Plastin 3 protects against spinal muscular atrophy (SMA) - the first fully protective modifier of a Mendelian disorder. *B. Wirth[1,2], S. Kröber[1,2], M. L. McWhorter[3], W. Rossoll[4], S. Müller[2], M. Krawczak[5], G. J. Bassell[4], C. E. Beattie[3], G. E. Oprea[1,2]* 1) Inst. of Human Genetics, Univ. of Cologne, Cologne, Germany; 2) Center for Molecular Medicine Cologne, Univ. of Cologne, D-Cologne; 3) Centre for Molecular Neurobiology, The Ohio State Univ., Columbus, OH; 4) Emory Univ. School of Medicine, Dept.of Cell Biology, Atlanta, GA; 5) Inst. of Medical Informatics and Statistics, Christian-Albrechts Univ. of Kiel, D-Kiel.

Homozygous deletion of SMN1 causes spinal muscular atrophy (SMA), the most frequent genetic cause of early childhood lethality. In rare instances, however, individuals are fully asymptomatic despite carrying the same SMN1 mutations and the same number of SMN2 copies as their affected siblings, thereby suggesting the influence of modifier genes. By comparing the transcriptom from lymphoblastoid cell lines between unaffected and affected SMN1-deleted siblings, we identified plastin 3 (PLS3; Xq23) to be highly expressed in all unaffected but not in the affected counterparts. PLS3 expression in blood turned out to be a rare variant, occurring only in 5% of controls. We found that PLS3 is highly expressed in spinal cord, associates with SMN, and together are part of a large multiprotein complex in spinal cord. The two proteins are present at similar subcellular locations in primary motor neurons and increase in expression during neuronal differentiation. PLS3, as an actin bundling protein, influenced the F-actin levels known to be involved in axonal outgrowth and guidance. PLS3 knock-down severely affected axonal growth, whereas its overexpression induced axonal growth. Most importantly, over-expression of PLS3 rescued the axonal growth defects caused by reduced SMN levels in neuronal differentiated PC12 cells, in primary motor neurons of SMA mouse embryos and in an in vivo zebrafish SMA-model. Our data strongly support the view that the involvement of SMN in axonal outgrowth and pathfinding is the major pathogenic defect in SMA. The results may help to identify novel targets for SMA therapy. This discovery signifies a major breakthrough in medical genetics in that it represents the first report ever of a fully protective modifier for a Mendelian disorder in humans.

Associated malformations in cases with congenital diaphragmatic hernia. *C. Stoll, Y. Alembik, B. Dott, M. P. Roth*
Genetique Medicale,Faculte de Medecine,Strasbourg, France.

The etiology of congenital diaphragmatic hernia (CDH) is unclear and its pathogenesis is controversial. Because previous reports have inconsistently noted the type and frequency of malformations associated with CDH, we assessed these associated malformations ascertained between 1979 and 2003 in 334,262 consecutive births. Of the 115 patients with the most common type of CDH , the posterolateral, or Bochdalek-type, hernia , 70 (60.8%) had associated malformations. These included patients with chromosomal abnormalities (21, 30.0%); non-chromosomal syndromes including Fryns syndrome, fetal alcoholism syndrome, De Lange syndrome, CHARGE syndrome, Fraser syndrome, Goldenhar syndrome, Smith-Lemli-Opitz syndrome, multiple pterygium syndrome, Noonan syndrome, and spondylocostal dysostosis; malformation sequences including laterality sequence, and ectopia cordis; malformation complexes including limb body wall complex and non syndromic multiple congenital anomalies (MCA) (30, 42.9%). Malformations of the cardiovascular system (42, 27.5%), urogenital system (27, 17.7%), musculoskeletal system (24, 15.7%), and central nervous system (15, 9.8%) were the most common other congenital malformations. We observed specific patterns of malformations associated with CDH which emphasizes the need to evaluate all patients with CDH for possible associated malformations. Geneticists and pediatricians should be aware that the malformations associated with CDH can be often classified into a recognizable malformation syndrome or pattern (57.1%).

Systematic resequencing of the coding exons of the X chromosome in X-linked Mental Retardation. *M. Stratton*
Cancer Genome Project, Wellcome Trust Sanger Inst, Hinxton, Cambs, United Kingdom.

Mental retardation (MR) affects 1-3% live births and has both genetic and non-genetic causes. A proportion of cases with genetic abnormalities are attributable to mutations of genes on the X chromosome. Although several X-linked MR (XLMR) genes have been reported, identification of more by conventional approaches is problematic because mutations of many genes cause MR and their associated phenotypes are similar. Here, we have implemented a new strategy in which the coding exons of most X chromosome genes (720/850, ~1Mb DNA per sample) have been systematically resequenced for disease-causing variants in individuals from more than 200 XLMR families. The strategy has yielded several new XLMR genes. However, many families remain to be explained. The study also indicates that loss of function of ~1% of X-genes is compatible with apparently normal existence. To our knowledge, this is the largest resequencing study to identify human disease genes thus far conducted. The results highlight issues that will be faced in the future by whole genome screens for rare disease-causing variants.

Mosaicism for a structural abnormality with 12p duplication. *S. Wenger[1], C. Cutenese[1], M. Hummel[2]* 1) Dept Pathology, West Virginia Univ, Morgantown, WV; 2) Dept Pediatrics, West Virginia Univ, Morgantown, WV.

At 38 weeks gestation, a female infant was delivered to a 19 year old mother with a history of polyhydramnios and gestational diabetes. The infant had a VSD, anal fistula, hypertension and mild hypotonia. She was hospitalized for 3 ½ weeks due to breathing problems. She was seen at 6 months of age by genetics and was noted to have minor dysmorphic features and developmental delays. She showed significant developmental delay and hypotonia at 10 months of age. Her karyotype was 46,XX,dup(12) (p12.2p13.3)[5]/46,XX[15]. The additional material on 12p was confirmed to be a duplication using TEL and subtelomere FISH probes. The patient has facial features similar to tetrasomy 12p, or Pallister-Killian syndrome, including the sparse temporal hair. Only about two dozen cases with mosaicism involving an autosomal structural abnormality have been reported in the literature, which have included duplications, deletions, insertions, isochromosomes and derivative chromosomes. The presence of both a normal cell line and structural abnormality indicates a mitotic error. The phenotype of 12p duplication is similar to that seen for tetrasomy 12p. Since our patient has significant developmental delay, most likely the mitotic duplication error occurred early during development. One of the characteristics of tetrasomy 12p is loss of the abnormal cell line in peripheral blood. Since the clinical consequences of duplication 12p are not as dramatic as tetrasomy 12p, the structurally abnormal cell line in our patient may not have as great of a growth disadvantage, resulting in the presence of the abnormal cell line in peripheral blood. We would suspect that the structurally abnormal cell line in our patient, similar to tetrasomy 21p, is more prevalent in fibroblasts, and will completely disappear from peripheral blood over time.

Reproductive Genetic Counseling in Patients with Complex Chromosomal Rearrangement. *N. Takeshita[1,2,3], Y. Katagiri[1,2,3], Y. Fukuda[1,3], M. Kitamura[1,3], Y. Iizuka[1], H. Takano[1], Y. Ishihara[1], T. Taniguchi[1], A. Oji[1], M. Saigusa[1], H. Hayashi[1], Y. Sasaki[1,3], S. Watanabe[1,3], Y. Matsue[1,3], A. So[1,3], C. Aoki[1], Y. Yao[1], T. Maemura[1], M. Tanaka[1], M. Morita[1]* 1) Department Obstetrics and Gynecology, Toho University, School of Medicine, 6-11-1 Omori Nishi, Ota-Ku, 143-8541, Tokyo, Japan; 2) Division forClinical Genetics, Toho University, School of Medicine, 6-11-1 Omori Nishi, Ota-Ku, 143-8541, Tokyo, Japan; 3) Center for Reproductive Medicine and Infertility, Toho University, School of Medicine, 6-11-1 Omori Nishi, Ota-Ku, 143-8541, Tokyo, Japan.

IntroductionThe first birth of a child by in vitro fertilization (IVF) in 1978 was a landmark event in the field of medicine. In the three decades since, there has been remarkable progress in assisted reproductive technology (ART). In the three decades since, there has been remarkable progress in assisted reproductive technology (ART). Recently, we conducted reproductive genetic counseling for a couple with a complex chromosomal rearrangement (CCR) that had five cleavage points involving four chromosomes 1, 6, 9 and 14. Here we present this case with some discussion regarding key points and matters to be considered for such counseling.This is a case report of reproductive genetic counseling for a couple with a CCR. The patient was a man aged 34 who was diagnosed with oligozoospermia. **Results** The results of chromosome analysis revealed that the chromosomal karyotype of the CCR in this patient was found to be 46, XY, der (1) (1qter1p13.3::9q22.19qter), der (6) (6pter6q15::?1p13.3?1p31.2::14q3114qter), der (9) (9pter9q22.1::6q156qter), der (14) (14pter14q31::1p31.21pter). **Conclusions** In patients with CCR, it is important to provide the patient with sufficient information and gain understanding of the pathogentic mechanism that underlies this genetic error. Pregnancy is dependent on the presence of phenotypic aberration. It is not infrequent that miscarriage occurs due to a high probability of unbalanced chromosome constitution even if pregnancy is confirmed. Furthermore, it may be difficult to establish an accurate preimplantation genetic diagnosis (PGD) in CCR patients.

An Integration of Genome-Wide Association (GWA) and Microarray Expression Profile to Accelerate the Discovery of New Candidate Genes. The Framingham Osteoporosis Study. *Y. Hsu[1], S. Demissie[2], K. Cho[2], Y. Zhou[2], E. Bianchi[3], SL. Ferrari[3], LA. Cupples[2], D. Karasik[1], DP. Kiel[1]* 1) Hebrew SeniorLife and Harvard Med. Sch., Boston, MA; 2) Biostat., BU, Sch Public Health, Boston, MA; 3) Div. of Bone Dis., Geneva Univ. Hosp., Geneva, Switzerland.

GWA approach is a powerful tool for identifying susceptibility genes of common diseases and providing novel targets for potential therapies. While a powerful method, GWA is not without challenges. Critical to success is to minimize the false association signals due to testing large number of markers. To overcome the challenge, we integrated mouse gene expression atlas with GWA study to identify and prioritize subsets of novel candidate genes for further evaluation. We conducted a GWA study using the Affymetrix 550K SNP chips to localize susceptibility genes for bone health phenotypes (bone density and hip geometric indices) in 2073 women and 1554 men (677 extended pedigrees) from Framingham cohorts. Family-based association test weighted by rank order of population-based linear mixed effect model statistics was used to estimate the genome-wide significance. The 3000 most significant SNPs from each trait were further evaluated in 2650 men and 5850 women from 2 independent studies (Rotterdam and the UK Twins Studies). To test whether associated genes were expressed in bone, we measured mRNA of PTH-differentiated primary osteoblasts, profiled bone cell expression from aging mice, and examined an embryonic mouse microarray database for in situ localization. Several novel candidate genes were found to be expressed in osteoblasts and genome-wide significantly associated with bone traits (i.e. PTPRD, SLC16A4, and PRKG1 genes). In addition, a gene-set enrichment analysis by Gene Ontology for the genes selected from the most significant SNPs suggested significant clustering of genes involved in CNS (nervous) development. In conclusion, our results reveal novel candidate genes and pathways to further elucidate the etiology of osteoporosis. To complement the leads from GWA studies, integration of expression profile allow effectively exploring the underlying mechanisms by which gene products act to alter disease susceptibility.

Association of Angiotensin-converting enzyme (ACE) gene insertion-deletion polymorphism with spondylarthropathies. *M. Z. Haider[1], D. K. Shehab[2], K. Al-Jarallah[2], A. M. Al-Awadhi[2], A. Al-Herz[3], I. Nahar[4]* 1) Pediatrics, Fac. of Medicine, Kuwait University, Safat, Kuwait; 2) Medicine, Fac. of Medicine, Kuwait University, Safat, Kuwait; 3) Medicine, Amiri Hospital, Kuwait; 4) Medicine, Mubarak Al-Kabeer Hospital, Kuwait.

Low back pain (LBP) is a common medical problem in which an interaction between genetic and environmental factors predisposes individuals even at an early age. Inflammatory back pain or spondylarthropathies include ankylosing spondylitis (AS), psoriatic arthritis(PSA), reactive arthritis enteropathic and undifferentiated arthropathies. Angiotensin-converting enzyme (ACE) plays an important role in circulatory homeostasis, physiology of vasculature and inflammation. The insertion-deletion (I/D) polymorphism of the ACE gene determines the plasma and tissue levels of ACE especially in the synovial fluid. The aim of this study was to investigate an association between ACE gene I/D polymorphism and inflammatory back pain (spondylarthropathies) secondary to ankylosing spondylitis (AS), psoriatic arthritis, inflammatory bowel disease and undifferentiated spondylarthropathies. The prevalence of ACE gene I/D polymorphism genotypes was determined in 63 patients with inflammatory back pain by polymerase chain reaction (PCR) and compared with that in 111 healthy controls. Of the 63 patients studied, 45 (71.4%) were with AS. 13 (20.6%) were with PSA, 4 (6.3%) were with reactive arthropathy and 1 (1.6%) manifested undifferentiated arthropathy. There were 43 males and 20 females. Mean age of patients was 39.0 11.36 years, age at onset of spondylarthropathy was 27.7 7.49 years and disease duration was 10.3 7.74 months. The ACE gene polymorphism showed an overall significant difference between patients and controls ($p = 0.05$). The incidence of heterozygous ID genotype was significantly higher in patients than in the controls 30.2% vs. 16.2% ($p = 0.01$), and DD genotype was more prevalent in controls 66.7% vs. 49.2% ($p = 0.01$). This study showed a significant association of the ACE gene I/D polymorphism with spondylarthropathy.

A large-scale survey of genetic copy number variations among Han Chinese residing in Taiwan. *C.-H. Lin[1], L.-H. Li[2], S.-F. Ho[2], T.-P. Chuang[2], J.-Y. Wu[2], Y.-T. Chen[2], C. S.-J. Fann[1,2]* 1) Department of Life Sciences and Institute of Genome Sciences, National Yang-Ming University, Taipei, Taiwan; 2) Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan.

Copy number variations (CNVs) have recently been recognized as an important structural variation in the human genome. CNVs affect expression of corresponding genes, and thus may contribute to certain phenotypic differences. The copy number inferring tool (CNIT) is an effective hidden Markov model-based algorithm for estimating allele-specific gene copy number and predicting chromosomal alterations from single nucleotide polymorphism microarrays. Using HapMap, we analyzed CNV information from 270 multi-ethnic individuals to determine the parameters of the CNIT algorithm and then applied the parameters to 300 unrelated Han Chinese residing in Taiwan. Under very restricted selection criteria, 230 regions having CNVs were identified in the Han Chinese population; 64 displayed greater than 1% minor allele frequency. The average size of the CNV regions was 322 kb (ranging from 1.48 kb to 5.68 Mb) and covered a total of 2.47% of the human genome. One hundred ninety-six of the CNVs were simple deletions and 27 were simple amplifications; 130 of the CNVs (56.52%) were reported previously. There were 449 genes and 5 microRNAs within these CNV regions and some of these genes are known to be associated with diseases. These CNVs are characteristic of Han Chinese populations and should be taken into consideration when genetic studies are conducted.

Possible implication of mRNA folding on the translation of FMR1 transcript on FMRP levels among normal and premutation carriers. *E. Peprah, W. He, E. Allen, T. Oliver, S. Sherman* Human Genetics, Emory University, ATLANTA, GA.

Fragile X syndrome (FXS) is the most common form of inherited mental retardation. FXS is caused by a trinucleotide repeat expansion in the 5 untranslated region (UTR) of the Fragile X mental retardation-1 (FMR1) gene. The expansion of CGG repeats can be grouped into categories based on the CGG repeat length and stability of the sequence. Normal individuals (6-44 repeats), intermediates (45-54 repeats), premutation carriers (55-199 repeats) and full mutations (>200 repeats). Alleles with >200 repeats lead to methylation of the regulatory region of FMR1, which induces transcriptional silencing. The absence of the FMR1 protein (FMRP) leads to FXS. Intriguingly, previous data have shown that FMR1 premutation carriers have increased FMR1 mRNA levels with decreased FMRP levels. The decreased levels of FMRP are thought to be due to translational inefficiency of the FMR1 transcript due to mRNA folding of the 5 UTR which contains the CGG repeats. To determine if there is a repeat length threshold for this effect we examined 31 males with repeats ranging from 29 to 110. For transcript and protein analysis, buffy coat samples were collected from fresh venous blood samples. Using quantitative protein assay, we confirmed the significant negative association between repeat length and FMRP. This association appeared to be linear, however we observed an increase in FMRP among repeat size ranges of 80-89. We conducted MFOLD analysis to better elucidate 5 UTR folding structures within each repeat size range. This increase could possibly be explained via the folding of the 5 UTR of the transcript. These data support a model of increasing inefficiency of protein translation based on increasing repeat size.

Stroke in Fabry Disease Frequently Occurs Before Diagnosis and in the Absence of Other Clinical Events: Natural History Data from the Fabry Registry. *K. Sims[1], J. Politei[2], M. Banikazemi[3], P. Lee[4]* 1) Center for Human Genetic Research and the Department of Neurology, Massachusetts General Hosp, Boston, MA; 2) Neurology Service, Juan Fernandez Hospital, Buenos Aires, Argentina; 3) Departments of Neurology and Pediatrics, New York University School of Medicine, New York, NY; 4) Charles Dent Metabolic Unit, National Hospital for Neurology & Neurosurgery, Queen Square, London, UK.

Strokes are a common and serious clinical manifestation of Fabry disease, an X-linked lysosomal storage disorder caused by deficiency of alpha-galactosidase A activity. A total of 138 patients of 2446 in the Fabry Registry (86 of 1243 males [6.9%] and 52 of 1203 females [4.3%]) experienced a stroke before starting enzyme replacement therapy. The median age at first stroke was 39.0 years in males and 45.7 years in females. The majority of patients (70.9% of males and 76.9% of females) had not experienced a renal or cardiac event before their first stroke. Fifty percent of males and 38.3% of females experienced their first stroke before they were diagnosed with Fabry disease. Thirty patients (21 males and 9 females) had strokes at age <30 years with a median age of 25.8 years in males (N=21) and 24.1 years in females (N=9). Two patients had strokes during their teenage years (a 13.8 year-old male and a 19.8 year-old females). 86.8% had ischemic strokes, but 13 of 77 males (16.9%) and 3 of 44 females (6.9%) had hemorrhagic strokes, among those for whom a stroke type was reported. Sixty percent of males and 25.5% of females exhibited stage 3-5 chronic kidney disease after their first stroke, based on the most recent eGFR data. 66.1% of males and 59.5% of females had left ventricular hypertrophy at some point after their first stroke. Compared to non-stroke patients, those who had strokes were more likely to have had a TIA (36.2% versus 5.4%), an arrhythmia (32.6% versus 12.7%) or hypertension (52.9% versus 20.5%). All patients with Fabry disease, regardless of age or gender, should be monitored closely for possible cerebrovascular complications, as stroke can occur in the absence of other key signs of the disease.

Syndrome Association with Cleft Lip/Palate in Center West of Brazil Region. *R. L. L. Séllos* dentistry, Hospital Materno Infantil, Goiania, GO., Goiania, Brazil.

The Cleft lip and palate (CLP) are frequent congenital malformations. Its happens in the 1st quarter of gestation, for flaw in the development of the lateral and medium nasal processes with the processes maxillaries. Studies show that 70% of CLP happen in subjects no syndromics, while the remaining 30% are frequently associated to the syndromes and sequence, existing about 350 of these: syndrome Pierre Robin, syndrome of Van der Woude, syndrome Treacher Collins... In Brazil, is considered 280.000 bearers of FLP, appearing 5.800 new cases/year, with tax of 1:650 new born. In Goiânia - Goiás, there is the Center of Rehabilitation of Cleft Lip and Palate (CERFIS), reference of the State, about 3.000 registered patients. Objective:, The objectives of the study went to describe the population assisted in this center in the period 1998-2004, and to identify the occurrence of syndromes associated with CLP. Methodology :, The sample totaled 1257 handbooks of patient with of CLP in the period. The studied variables were gender, hereditary, consanguinity, occurrence of syndromes associated to the cleft and classification of the cleft. The project was approved by the Committee of Ethics in Research of Hospital Materno Infantil. Results:, Of the 1.257 patients of CLP of the study, (46,7%) they were female and (53,3%) of the male. The family history of CLP was registered in 227 handbooks, 41 registrations referred consanguineous marriages,. Syndromes and/or sequences associated to CLP happened in 51 cases. Being the larger frequency of cases with Pierre Robin's sequence (60,8%). The classification of the clefttt: Cleft Lip 27%, Cleft Lip an Palate 43%, Cleft palate 27,7% and Rare fissures of the face 2.2% Conclusions:, The present study has shown that it would be appropriate to continue the investigation of patients attending CERFIS in order to characterize the environmental and genetic factors associated with the etiology of cleft Such information could be employed to improve the assistance given to patients and consequently the treatment applied.

Haploinsufficiency of FOXP1 is associated with Chiari I malformation and speech/language disorder. *O. Abdul-Rahman[1], H. Zimmerman[1], N. Justice[2], C. Lese-Martin[2]* 1) Dept Pediatrics, Univ Mississippi, Jackson, MS; 2) Dept Human Genetics, Emory University, Atlanta, GA.

We report on the first case of a patient with haploinsufficiency of FOXP1. The patient did not walk until 16 months of age. He was able to imitate sounds, but had no words. Hearing was normal. He uses sign language. Physical exam at age 3 showed normal growth. He had a broad forehead, hypertelorism, downslanting palpebral fissures, ptosis, short nose, full nasal tip, smooth philtrum, and down-turned mouth. MRI showed a Chiari I malformation. CGH detected a 1Mb deletion at 3p13. Parental analysis confirmed the deletion was de novo. The deletion involved 70,549,922 bp to 71,570,262 bp (hg 17) and FOXP1 is the only known gene in this region. FOXP1 is a transcriptional regulator that binds DNA through a winged-helix domain and influences gene expression through a zinc-finger and leucine zipper motif. This feature identifies it as a member of the forkhead family that include four members, FOXP1-4. Mutations in FOXP2 have been associated with speech-language disorder 1. Mutations in FOXP3 cause X-linked immunodysregulation, polyendocrinopathy, and enteropathy. In humans, FOXP4 expression is noted to be reduced in some tumors. Studies of FOXP1 expression suggest tumor suppressor activity and roles during embryonic expression in the nervous system. Abnormalities in FOXP1 expression are seen in colon, stomach, and prostate tumors in addition to B-cell lymphomas. Developmental expression studies of FOXP1 in zebrafish demonstrate increased signals in the midbrain, hindbrain, and anterior spinal cord. The presence of a Chiari I malformation in our patient, believed to be caused by dysgenesis of para-axial mesoderm in the developing hindbrain, is likely related to deficient FOXP1 expression during embryogenesis. Additionally, SPCH1 is a result of reduced FOXP2 expression in the perisylvian frontal and temporal regions important for auditory vocal learning and speech development. Songbird and human fetal brain analysis supports overlapping expression of both FOXP1 and FOXP2 in structures important for learned articulation, which may explain the speech/language phenotype in this patient.

Cellular impact of *RAI1* haploinsufficiency: *RAI1* functions through specific pathways to regulate growth, metabolism, and circadian rhythm. *S. Girirajan[1,2], H. T. Truong[3], C. L. Blanchard[3], S. H. Elsea[2]* 1) Department of Genome Sciences, University of Washington, Seattle, WA; 2) Depts. of Human and Molecular Genetics and Pediatrics, Virginia Commonwealth University, Richmond, VA; 3) School of Biomedical Sciences, Charles Sturt University, Wagga Wagga, NSW, Australia.

Smith-Magenis syndrome (SMS) is a complex syndrome characterized by a constellation of ~30 features that include sleep disturbance, craniofacial defects, neurological and behavioral anomalies, and variable systemic features. SMS is caused by a 17p11.2 deletion encompassing the retinoic acid induced 1 (*RAI1*) gene or by mutation of *RAI1*. While haploinsufficiency of *RAI1*, a known transcription factor, is the major cause of SMS, its exact functional pathways are not known. We hypothesized that *RAI1* acts through specific cellular pathways involving several downstream targets which are altered by *RAI1* haploinsufficiency. To identify genes in these *RAI1*-mediated pathways, we performed genome-wide gene expression analysis on cells haploinsufficient for *RAI1*. Human embryonic kidney (HEK293T) cells with RNA-interference-based ~50% knockdown of *RAI1* and lymphoblastoid cell lines created from SMS patients were utilized. Genome-wide gene expression profiling using microarrays showed that ~60 genes were upregulated and ~200 genes were downregulated due to *RAI1* haploinsufficiency. Real-time qRT-PCR confirmed the gene expression profile in knockdown HEK293T cells. Lymphoblastoid cell lines obtained from SMS patients confirmed the altered expression pattern in downstream genes that are involved in growth signaling and insulin sensitivity (*INSIG1, PIK3R1, ZNF236*), circadian activity (*NR1D2*), neuronal differentiation (*ZIC1, PSEN2, RXRb, SMA4, CLN8, NF1, MLL*), lipid biosynthesis and fat mobilization (*LIPE, HMGCS1*), skeletal development (*GLI3, PSTPIP2, ANKH*), behavior (*SCN12A*), gene expression (*SPTBN1, POLDIP3, PPP1R14D, ADD3*), cell cycle regulation (*RUNX1T1, AKR7A3, FBLN1, ZNF236*), and recombination (*RAD51*). Our study suggests that *RAI1* functions through several genes in specific pathways regulating various biological processes, that when disrupted result in the phenotypic effects observed in Smith-Magenis syndrome.

Health Technology Assessment of Gene Expression Profiles for the Prognosis of Recurrence of Primary Breast Cancer. *A. P. Lea, T. L. Rogstad, S. Levine* Hayes Inc., Lansdale, PA.

Objectives: A number of prognostic gene expression profiles (GEPs) have been developed to help identify patients most likely to benefit from systemic adjuvant chemotherapy following surgery for the treatment of early-stage breast cancer. A comparative evidence-based health technology assessment of 5 GEPs was performed focusing on their use to estimate the risk of recurrence of primary unilateral breast cancer in women. **Methods:** The ACCE model that was developed by the Centers for Disease Control and Prevention (CDC) was utilized for the assessment of genetic tests. A strict definition of clinical validation was also used: the assay methods to perform the test had to be the same as used in the study that derived the gene expression test, and also the tumor samples used for validation had to be independent of those included in the study that derived the test. The tests included in this assessment were the 2-gene ratio, the 5-gene panel, the 21-gene panel, the 70-gene panel and the 76-gene panel. Conclusions were based only on published data. **Results:** For most of the 5 GEPs, there were little analytical validity data available. Most of the research into these 5 GEPs focused on establishing clinical validity. Using conservative criteria, clinical validity was found to have been demonstrated for the 21-gene, 70-gene and 76-gene panels, but not the two-gene ratio or the 5-gene panel. No published prospective studies examining the clinical utility of any of the 5 GEPs were identified. **Conclusions:** Given the lack of published prospective studies on the clinical utility of any of the 5 GEPs, a clinical benefit of using these gene expression tests has yet to be established. Accordingly, despite the widespread use of some of these tests, from an evidence-based medicine perspective, none of these tests can be unequivocally recommended for clinical use. Results of ongoing major studies (MINDACT and TAILORx) will be essential in determining the clinical utility of these gene expression tests and defining any impact on patient outcomes.

Congenital Inborn Errors of Metabolism. 15 Years Experience at the Hospital Para el Niño Poblano(HNP), Mexico. *J. M. Aparicio[1,7], M. L. Hurtado[2], M. P. Barrientos[3], V. H. A. Leon[4], M. S. R. Gutierrez[5], M. P. A. Gallegos[6], H. O. Chavez[7], J. G. Vega[7], S. M. Chatelain[1]* 1) Dept Genetics; 2) Cytogenetics; 3) Endocrinology; 4) Pediatrics; 5) Clinical Laboratory, Hospital para el Nino Poblano, Puebla,; 6) Molecular Genetics, CIBO IMSS Guadalajara Jalisco,; 7) Estomatology Faculty, Benemerita Universidad Autonoma de Puebla, Mexico.

INTRODUCTION. Inborn errors of metabolism (IEM) comprise a large class of genetic diseases involving disorders of metabolism. Most of them are due to defects of single genes that code for enzymes that facilitate conversion of various substances called substrates. In most of the disorders, problems arise due to accumulation of substances which are toxic or interfere with normal function, or reduced ability to synthesize essential compounds. Inborn errors of metabolism are now often referred to as congenital metabolic diseases or inherited metabolic diseases, being both terms considered similar. The IEM are clinical identificables diseases mentioned by Garrod in 1908. Consanguinity was an importan issue if inheritance background was observed. CASES REPORTED. 2 370 patients were studied at the hospital during a 15 years period. All clinical patients with polymalformed muscle esqueletal and skull dismorfies were included in the study. 102 patients (4%) were found to be positive for IEM. RESULTS. From these 102 patients with IEM, 42 patients had amino acid, 2 mitochondrial and 43 carbohydrates alterations. 15 of these patients had mucopolysaccharidosis, from these 10 were diagnosed as Hurler, 2 as Hunter and 3 as Morquio syndromesFigure 1. CONCLUSIONS. The congenital Inborn Errors of Metabolism (IEM) are inherited diseases with a Mendelian autosomic recessive inheritance risk. A gen alteration produces an enzymatic defect with a unique biochemical alteration. Most of these genetic defects clinical manifestations are observed in children, were neonatal metabolic test are important. Actually, the IEM are defined as monogenic inherited diseases or mendelians, due to a metabolic error for a protein or enzime absence. It might be incompatible with the patient life and some times if the patient lives it will modify its quality life (Fenilketonuria).

Freeman Sheldon syndrome. A case report from the Hospital Para el Niño Poblano, Mexico. *M. Barrientos[1], J. M. Aparicio[2,5], N. C. O. Gil[3,5], W. B. San Martin[3], R. A. E. Garcia[3], E. M. Landini[4], S. M. Chatelain[2]* 1) Endocrinology; 2) Genetics; 3) Estomatology; 4) Orthopedics, Hospital para el Niño Poblano, Puebla, puebla, Mexico; 5) Estomatology Faculty, Benemerita Universidad Autonoma de Puebla.

INTRODUCTION. Freeman Sheldon syndrome (FSS) is characterized by talipes equinovarus, camptodactyly, scoliosis, abnormalities of the muscles of the eye, microstomia, high-arched palate, attenuated movement of the muscles of facial expression and other primary anomalies involving the musculoskeletal system. On the whole, DA1 is the least severe; DA2B is more severe with additional features that respond less favourably to therapy. DA2A (FSS) is the most severe of the three, with more abnormalities and greater resistance to therapy. FSS has been described as a type of congenital myopathy. In March 2006, Stevenson et al. published strict diagnostic criteria for distal arthrogryposis type 2A (DA2A) or Freeman-Sheldon syndrome. These included two or more features of distal arthrogryposis: microstomia, whistling-face, nasolabial creases, and 'H-shaped' chin dimple. CASE REPORTED. A 3 years old female patient, was diagnosed as FSS, due to her clinical phenotype, short stature, whistling-face, nasolabial creases, 'H-shaped' chin dimple, microstomia, high-arched palate, attenuated movement of the muscles of facial expression. Distal arthrogryposis, and camptodactyly. Congenital cardiopaty, auricular communication, was also observed. CONCLUSIONS. Patients with FSS must have early craniofacial and orthopedic evaluation since tey have craniofacial and musculoesqueletic malformations. Correction is indicated to improve function or aesthetics. Although some surgical procedures have suboptimal outcomes, due to the muscular pathology of FSS. Genetic counseling is also important, since FSS has been associated to either autosomal dominant, most often demonstrated or autosomal recessive inheritance. FSS is caused by genetic changes. Krakowiak mapped the distal arthrogryposis multiplex congenital (DA2B) gene, a syndrome very similar in phenotypic expression to classic FSS, to 11p15.5-pter.Early intervention leads the possibility to minimize developmental delays and improve basic functions.

Genetic counseling in carriers of reciprocal chromosomal translocations involving two autosomes. *H. Pour-Jafari[1,2], M. Farimani[2], S. Ghahramani[3], M. Hashemzadeh Chaleshtori[4], B. Pour-Jafari[2]* 1) Molecular Medicine & Genetics, School of Med., Hamadan Univ Med Sciences, Hamadan, Hamadan, Iran; 2) Research Centre for Molecular Medicine, Hamadan University of Medical Sciences, Hamadan, Iran; 3) Genetic Laboratory, Sh. Beheshti Hospital, Hamadan, Iran; 4) Molecular Genetic Research Center, Shahre Kord, Iran.

One of the main genetic causes involve in the pathogenesis of recurrent abortion is parental chromosomal abnormalities. The central concept in genetic counseling with such families is to estimate the probability of recurrence of unfavorable pregnancy outcomes. The main questions that consultants usually ask are: why this happened? What is the risk to be done again? Our cases were two families with repeated miscarriage. The pedigrees were drawn, the chromosomes of couples were studied and estimation for recurrent risk was done. We tried to answer those two main questions and clear the results for them. Parental chromosome abnormalities were found after karyotyping with GTG technique at 450 band resolution, revealing 46 chromosomes with balanced translocation of autosomes in one of the partner in both families. Recurrent risk was estimated as 1/6 for their future pregnancies in each family. Couples in which one partner is the carrier of such balanced translocation have increased risks of infertility, recurrent abortion, and delivery of chromosomally abnormal offspring. Genetic counseling of such couples therefore presents a unique challenge and should be considered in dealing with such families.

A Survey of (CAG)n Repeats Causing the Juvenile Huntington Disease in an Iranian Family with Four Affected Members. *B. Pour-Jafari[4], M. Mazdeh[2], A. Ghaleiha[3], H. Pour-Jafari[1,6], M. Houshmand[5], F. Talebzadeh[6], M. Rostami[5]* 1) Mol Med & Genetics, School of Medicine, Hamadan Univ Med Sciences, Hamadan, Iran; 2) Neurology Dept, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran; 3) Psychiatry Dept, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran; 4) SKG Heart Center, Houston, TX; 5) Special Medical Center, Enghelab Ave., Ostad Nejat Allahi, #1, Tehran, Iran; 6) Research Center for Molecular Medicine, Hamadan University of Medical Sciences, Hamadan, Iran.

Huntington's disease is a rare inherited neurological disorder. HD is caused by a trinucleotide repeat expansion (CAG)n in the gene coding for Huntingtin (Htt) and is one of several polyglutamine diseases. Huntington's disease's most obvious symptoms are abnormal body movements called chorea and a lack of coordination, but it also affects a number of mental abilities and some aspects of personality. These physical symptoms occur in a large range of ages, with a mean occurrence in a person's late forties/early fifties. If the age of onset is below 20 years then it is known as Juvenile HD. Huntington's disease is autosomal dominant, needing only one affected allele from either parent to inherit the disease. Although this generally means there is a one in two chance of inheriting the disorder from an affected parent, the inheritance of HD and other trinucleotide repeat disorders is more complex. Our case was an Iranian family with four affected sibs (2 daughters and 2 brother). They had 4 affected and 5 normal male children. There was no any other case in their family history. The onset age of the disease in our case family was 20 to 25 years. Their parents were unaffected and nonconsanguineous. Analysis of pathogenic (CAG)n repeat region of the HD gene for the affected members have showed an expansion allele with 46, 50, 46 and 44 repeats in four affected sibs. These results indicate that molecular analysis after clinical diagnosis for HD inclusions facilitates the pathological evaluation of HD and enhances its accuracy.

Differential response to Galsulfase therapy in brothers with mucopolysaccharidosis VI (Maroteaux-Lamy). *A. Scheuerle* Tesserae Genetics, Dallas, TX.

Mucopolysaccharidosis VI (MPSVI; Maroteaux-Lamy) is a recessive lysosomal storage disease due to mutations in arylsulfatase B (ARSB). Galsulfase enzyme therapy (ERT) was made available in 2005. Two brothers who began ERT at different ages and stages of disease demonstrate the advantages of early treatment. Case 1: 30 m/o BM. He delivered by caesarean section due to macrocephaly. Motor development was delayed. Language and cognitive development were normal. He had frontal bossing, chronic rhinorrhea, corneal clouding, decreased use of his left arm, and an unusual gait. Enzyme diagnosis of MPSVI was made (ASRB = 0.0 nmol/min/mg protein). He was begun on ERT at 33 months of age. A fall at home resulted in C3/C4 subluxation and spinal cord injury. He had cervical spine fusion, recovering well. He regained much of his motor function. At one year of therapy there was no measurable progression of disease and no adverse reaction to infusion. At 4.5 y/o he had physical features of severe MPS VI, but was cognitively normal. Urine glycosaminoglycans (GAG) went from 617.9g/mg creatinine to 200 without proteinuria. He has had an antibody response to the enzyme. Case 2: Newborn BM. Postnatal enzyme analysis confirmed ASRB = 0.0 nmol/min/mg protein. He began ERT at 5 weeks of age. At one year of therapy he had normal growth and development. Physical exam was normal except for scaphocephaly. Skeletal survey, head magnetic resonance imaging and echocardiogram were normal. At 20 m/o his weight was 30th centile, length 20th centile and head circumference 50th centile. His physical exam was normal except for mild frontal bossing. Urine GAG went from 214g/mg creatinine to 120.5 without proteinuria. At a year of treatment he had not mounted an antibody response to the enzyme. The cases show: 1) normal cognition even with severe physical disease, 2) efficacy of galsulfase to stabilize the phenotype, and 3) efficacy of galsulfase to prevent physical involvement when started presymptomaticly. It is likely that the younger brother would have had a more involved course if ERT had not been started early. This experience also supports the observation that younger children have a lower risk of anaphylactoid events and may tolerate the enzyme.

Genome wide admixture mapping identifies MYH9 as a major effect risk gene for focal segmental glomerulosclerosis. *C. Winkler[1], M. Smith[1], G. Nelson[1], D. Vlahov[2], B. Freedman[3], D. Vlahov[3], T. Olelsyk[1], J. Kopp[4], NIH Kidney Genetic Study and the Wake Forest ESRD Study* 1) Dept Molec Gen Epidemiology, SAIC, NCI-Frederick, Frederick, MD; 2) New York Academy of Medicine, New York, NY; 3) Section of Nephrology, Wake Forest School of Medicine; 4) Kidney Disease Section, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD.

The increased burden of chronic kidney disease and end stage kidney disease (ESKD) in populations of West African ancestry is only partially explained by socioeconomic status; the genetic basis remains unexplained. African Americans are at a 4-fold and 18-50-fold increased risk for idiopathic focal segmental glomerulosclerosis (FSGS) and HIV-1-associated nephropathies, respectively, compared to European Americans. Approximately 20 percent of ESKD is attributable to FSGS. To identify genetic variants predisposing to idiopathic and HIV-1 associated FSGS, we used a mapping by admixture linkage disequilibrium genome scan on 190 African American FSGS cases and 222 controls that identified a region of chromosome 22 with a significant genome-wide logarithm of the odds (LOD) score of 9.2 and a peak LOD of 13.6 that centers on *MYH9*. *MYH9* is both a positional and functional candidate gene as it is expressed in kidney podocytes, critical cells required for glomerular filtration. Three *MYH9* SNPs ($P=2x10^{-18}$ - $1x10^{-20}$ and OR=4.5 - 4.8, recessive) were most strongly associated with FSGS in African Americans (n=852); the *MYH9* association was confirmed in Europeans (346) for FSGS (OR=9, P=0.02, recessive), for all three SNPs. In an extension study, the same SNPs were also associated with hypertensive ESKD (n=433) (OR=2.2, $P=7x10^{-5}$, recessive for SNP rs4821481), but not with diabetic ESKD (n=476) in African Americans. The increased risk for FSGS and hypertensive ESKD among African Americans is substantially due to one or more *MHY9* genetic risk alleles frequent on African-origin haplotypes (frequency60%) but much less frequent on European-origin haplotypes (4%).

Current depression in LGI1 mutation carriers. *G. Heiman[1], R. Ottman[2]* 1) Dept Genetics, Rutgers Univ, Piscataway, NJ; 2) G.H. Sergievsky Center, Columbia Univ, NY, NY.

Depression is the most common comorbid psychiatric condition in individuals with epilepsy, but the cause is unknown. Explanations include an emotional reaction to a physical disorder, effects of seizures on the brain, and a shared genetic susceptibility. We addressed this problem by investigating depression in families with a known genetic cause of epilepsy, i.e., families with autosomal dominant partial epilepsy with auditory features (ADPEAF) with mutations in the leucine-rich, glioma inactivated 1 gene (LGI1). We administered a depression interview to 70 members of 8 families, each of which had a different mutation in LGI1. We classified family members into three groups: mutation carriers with epilepsy (n=25), mutation carriers without epilepsy (n=9), and non-carriers (n=36). Only two subjects, both of whom were mutation carriers with epilepsy (8%), met criteria for current *major depressive disorder*. Nine subjects met criteria for current *other depressive disorder*. The risk for current *other depressive disorder* was increased non-significantly in both mutation carriers with epilepsy (OR=2.10, 95% CI=0.42-10.51) and mutation carriers without epilepsy (OR=3.14, 95% CI=0.37-26.37), compared with non-carriers. Current depression symptom scores were significantly higher in mutation carriers with epilepsy than non-carriers (mean = 5.4 vs. 1.9, p=0.003). However, current depression symptom scores were not higher in mutation carriers without epilepsy than non-carriers (mean = 2.3 vs. 1.9, p=0.71). In this small study, current other depressive disorder (i.e., previous 2 weeks) was elevated in LGI1 mutation carriers both with and without epilepsy whereas current depressive symptoms were only increased in LGI1 mutation carriers with epilepsy. While the results for other depressive disorder are suggestive of a shared genetic susceptibility, analyses of current depressive symptoms suggest that the comorbidity of depression and epilepsy is related to having epilepsy. Future studies, using a larger sample and assessing lifetime rather than current depression, could help to clarify whether or not depression is an alternative manifestation of LGI1 mutations.

Novel Methods for Detecting Associations with Rare Variants for Common Diseases: Application to Analysis of Sequence Data. *B. Li, S. M. Leal* Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Although whole genome association studies using tagSNPs are a powerful approach for detecting common variants, they are underpowered to detect associations with rare variants. Recent studies have demonstrated that common diseases can be due to functional variants with a wide spectrum of allele frequencies ranging from rare to common. An effective way to identify rare variants is through direct sequencing. The development of cost-effective sequencing technologies enables association studies to use sequence data from candidate genes and in the future from the entire genome. Although methods used for analysis of common variants are applicable to sequence data, their performance may not be optimal. In this study it is shown analytically that the collapsing method, which involves collapsing genotypes across variants and applying a univariate test, is powerful for analyzing rare variants, while multivariate analysis is robust against inclusion of non-causal variants. Both methods are superior to analyzing each variant individually using univariate tests. In order to unify the advantages of both collapsing and multi-marker tests, we developed the Combined Multivariate and Collapsing (CMC) method and it is demonstrated analytically that the CMC method is both powerful and robust. The CMC method can be applied to either candidate gene or whole genome sequence data.

Identification of Skin Abnormalities in Osteogenesis Imperfecta Patients by Magnetic Resonance ImagingA Pilot Study. *E. M. Carter[1], C. L. Raggio[2], K. W. Fishbein[3], M. Kim[5], N. Pleshko[4], R. G. Spencer[6]* 1) Center for Skeletal Dysplasias, Hospital for Special Surgery, New York City, NY; 2) Pediatric Orthopaedics, Hospital for Special Surgery, New York City, NY; 3) National Institutes on Aging, NIH, Baltimore, MD; 4) Biomechanics, Exponent, Philadelphia, PA; 5) Mineralized Tissue Laboratory, Hospital for Special Surery, New York City, NY; 6) Nuclear Magnetic Resonance Unit, National Institute on Aging, NIH, Baltimore, MD.

Osteogenesis imperfecta (OI) is a genetic disorder characterized by bone fragility and frequent fractures. Diagnosis is based on clinical and radiological criteria and increasingly by genetic test results. We tested the hypothesis that magnetic resonance imaging (MRI) can detect skin abnormalities that correlate with OI genotype and phenotype. Our primary research objectives were to determine: 1. whether MRI can differentiate between skin from OI and control subjects; 2. whether nondestructive MRI analysis is supported by invasive but highly specific Fourier transform infrared spectroscopic imaging (FT-IRIS); and 3. whether there is a relationship between genotype, skeletal phenotype, and skin phenotype across patients of all ages. MRI analysis of 3-mm full-thickness forearm skin biopsies from OI (n=6) and non-OI control (n=2) subjects was performed, followed by FT-IRIS. MRI parameters, including T1, T2, and magnetization transfer (MT), were compared to FT-IRIS parameters characterizing dermal collagen. Initial findings showed clear differences in both MRI and FT-IRIS parameters between patients with OI and controls. Findings within the OI group correlated with the severity of clinical phenotype; epidermal and dermal layers were thinner in OI patients compared to controls with the degree of thinning correlating with the severity of OI phenotype. MRI revealed fat deposits within the dermis of OI skin only. FT-IRIS revealed differences in collagen orientation in the dermis of OI skin compared to controls. We conclude that MRI is sensitive to presence and severity of OI in human skin, as confirmed by FT-IRIS analysis. This supports the potential for developing an MRI approach for rapid non-invasive diagnosis of children with OI.

Treatment and Prevention of SMA through Newborn and Population Carrier Screening. *T. W. Prior[1], R. E. Pyatt[1], D. C. Mihal[1], T. Conlan[1], P. J. Snyder[1], B. Schmalz[1], L. Montgomery[1], K. Ziegler[1], S. Hashimoto[2], S. Garner[2], C. Noonan[2]* 1) Ohio State University; 2) Riverside Methodist Hospital, Columbus, Ohio.

The identification of SMA in newborns will allow these children enrollment into current clinical trials earlier, before irreversible motor neuron death, and allow for an accurate diagnosis, genetic counseling and family planning. A new chemistry for the detection of the common SMN1 deletion from newborn blood spots using a microbead array luminex platform was developed. We have currently completed the screening of 40,130 bloodspots (from the Ohio Department of Health) and 4 homozygous SMN1 deletions have been detected. SMN2 copy numbers were determined and indicated that 2 of the spots had 2 SMN2 (most consistent with type I SMA) and 2 had 3 SMN2 (most consistent with type II or III SMA). Our pilot study has demonstrated that screening for SMA can be technically accomplished on a large-scale newborn population basis. Currently, individuals with a family history of SMA are most often offered carrier testing. However, more broad-based population carrier screening is currently recommended for a number of genetic disorders. The goal of the Claire Altman Heine Foundation SMA population carrier screening pilot program is to identify couples at risk for having a child with SMA, to gain a better estimate of the carrier frequency, to determine the allele frequencies of the two copy SMN1 chromosomes and to study the practical, technical, cost effectiveness, and feasibility of large-scale screening for SMA carrier status. Formal genetic counseling services have been made available to everyone requesting this testing at the Ohio State University and Riverside Methodist Perinatal Centers. 187 individuals have currently been screened and 7 carriers have been identified. Six of the seven carrier spouses were tested and all exhibited noncarrier status. Our survey results indicate that although there is a general lack of familiarity with SMA, there is general interest in carrier screening and therefore population screening may be a means of reducing the prevalence of SMA. The findings of our work have significant implications for risk assessment and genetic counseling of SMA.

Comparison of BAC array-CGH data using formalin-fixed paraffin-embedded versus fresh frozen specimens in multiple myeloma and follow-up FISH. *P. Lennon[1], C. Perez[1], D. Sebastian[1], J. Abraham[1], P. Hu[1], D. Pierson[3], C. Williams[2], X. Zhang[3], P. Lin[3]* 1) Sch Health Sci, UT-MD Anderson Cancer Ctr, Houston, TX 77030; 2) Perkin Elmer BAC Array-CGH Core, UT-MD Anderson Cancer Ctr, Houston TX 77030; 3) Dept Hematopathology, UT-MD Anderson Cancer Ctr, Houston, TX 77030.

Introduction: Multiple Myeloma is an incurable disease involving neoplastic plasma cells, often harboring chromosomal aberrations. Some aberrations are implicated in neoplastic transformation, disease progression, and prognosis. Fresh bone marrow (FT) aspirates are considered most reliable for molecular genetic analysis; however, formalin fixed paraffin-embedded (FFPE) tissues are easily retrievable. Compared to conventional cytogenetics, array-CGH allows more sensitive detection of chromosomal abnormalities. Method: We analyzed seven paired samples of (FT) and (FFPE) bone marrow aspirate samples obtained from MM patients to determine the efficacy of aCGH using FFPE. Results: A total of 34 aberrations were identified of which 29/34 were found in both sample types, yielding 85% concordance. Nonrandom anomalies were observed including 7q+, 15q+, 19p+, 8p-, and 13q- of paired samples of at least 2 cases. We verified these results performing fluorescent in situ hybridization on FFPE and bone marrow smears using Williams probe for 7q+, and a home-brewed overlapping probe created with BACs (RP11-119K8 and RP11-61M22) to target 15q+. In addition, we analyzed FFPE samples of 3 cases of Monoclonal Gammopathy of Undetermined Significance (MGUS) by FISH to determine if 7q+ might be an early, possibly transforming event, and found that 1 of 3 cases did in fact carry 7q+. Conclusions: We conclude that array-CGH analysis can be effective using FFPE samples and is a sensitive method for identification of chromosomal aberrations. The non-random chromosomal anomalies found via array-CGH and validated via FISH are authentic, and some may represent early transforming events that affect disease progression in multiple myeloma. The other non-random changes detected by array-CGH should also be validated via FISH and examined in MGUS patients as well to determine their possible early arrival in this disease process.

Identification of multiple novel prostate cancer predisposition loci. *R. Eeles[1], Z. Kote-Jarai[1], D. Easton[2], J. Stanford[3], E. Ostrander[4], J. Schleutker[5], S. Ingles[6], D. Schaid[7], S. Thibodeau[7], T. Dork[8], D. Neal[9], W. Vogel[10], M. A. Kedda[11], E. John[12], G. Giles[13], W. Foulkes[14], P. Chappuis[15], K. Muir[16, 17], M. Guy[1], A. Amin Al Olama[2], The PRACTICAL Group & ProtecT Group & UKGPCS Study* 1) Cancer Genetics Unit, Inst Cancer Research, Sutton, United Kingdom; 2) Cancer Research UK Genetic Epidemiology Group, Strangeways Laboratory, Cambridge, United Kingdom; 3) Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, USA; 4) National Human Genome Research Institute, National Institutes of Health, Bethesda MD, USA; 5) Institute of Medical Technology, University of Tampere and Tampere University Hospital, Tampere, Finland; 6) Department of Preventive Medicine, University of Southern California Keck School of Medicine, Los Angeles CA, USA; 7) Mayo Clinic, Rochester, Minnesota, USA; 8) Hannover Medical School, Germany; 9) Surgical Oncology, Cambridge Research Institute, Cambridge, UK; 10) Institut für Humangenetik, Ulm, Germany; 11) School of Public Health and Institute of Health and Biomedical Innovation, Queensland, Australia; 12) Northern California Cancer Center, Fremont, California, USA; 13) Cancer Epidemiology Centre, The Cancer Council Victoria, Australia; 14) McGill University, Montreal, Canada; 15) Division of Genetic Medicine and Division of Oncology, University Hospitals of Geneva, Switzerland; 16) University of Nottingham UK; 17) Chulabhorn Cancer Research Centre, Thailand.

A genome wide association study in prostate cancer (PC)involving analysis of over 500 000 SNPs in 3748 blood samples from men with prostate cancer has found genetic variants on chromosomes 3, 6, 7, 10, 11, 19 and X are associated with PC risk. Blood DNA from 7370 PC cases and 5742 control men was analysed in a follow up study in 13 groups worldwide (The PRACTICAL Group). The per allele OR was 1.12 to 1.29 and combined risks were consistent with a multiplicative risk model. These and previous loci explain 16 per cent of familial risk of PC, and men in the top 10 per cent of the risk distribution have a 2.1 fold risk relative to general population rates. Genetic profiling of such variants will enable research studies in targeted PC screening.

Clinical spectrum of patients with SOS1 mutations ranges from Noonan syndrome to cardio-facio-cutaneous syndrome. *Y. Narumi[1,2], Y. Aoki[1], T. Niihori[1], M. Sakurai[3], H. Cavé[4], A. Verloes[4], K. Nishio[5], H. Ohashi[6], K. Kurosawa[7], N. Okamoto[8], H. Kawame[9], M. C. Addor[10], C. Vincent-Delorme[11], A. Coeslier-Dieux[12], M. Aoki[13], A. Guliyeva[1], T. Kobayashi[1], S. Kure[1], Y. Matsubara[1]* 1) Dept Med Genet, Tohoku Univ Sch Med, Sendai, Japan; 2) Div Med Genet, Gunma Children's Med Center, Shibukawa, Japan; 3) Dept Cardiovascular Surgery, Tohoku Univ Sch Med, Sendai, Japan; 4) Dept Genetics, Hôpital Robert Debré, Paris, France; 5) Seirei Hamamatsu General Hospital, Hamamatsu Hosp, Japan; 6) Saitama Childrens Med Ctr, Saitama, Japan; 7) Kanagawa Children's Med Ctr, Yokohama, Japan; 8) Osaka Med Ctr & Res Inst for Maternal & Child Health, Osaka, Japan; 9) Nagano Children's Hosp, Nagano, Japan; 10) Dept Med Genet, CHU Vaudois, Lausanna, Switzerland; 11) Dept Med Genet, CHRU de Lille, Lille, France; 12) CH d'Arras, Arras, France; 13) Dept Neurol, Tohoku Univ Sch Med, Sendai, Japan.

Noonan syndrome (NS) and cardio-facio-cutaneous (CFC) syndrome are characterized by cardiac defects, facial dysmorphism, ectodermal abnormalities and mental retardation. Mutations in PTPN11 and KRAS have been identified in patients with NS and those in KRAS, BRAF and MAP2K1/2 have been identified in patients with CFC syndrome. Recently, mutations in the son of sevenless gene (SOS1) have been also identified in patients with NS. To clarify the clinical spectrum of patients with SOS1 mutations, we analyzed 24 patients with NS, including three patients in a three-generation family and 30 patients with a CFC phenotype without PTPN11, KRAS, HRAS, BRAF and MAP2K1/2 mutations. We identified two SOS1 mutations in four NS patients, including three patients in the above mentioned three-generation family. In the patients with a CFC phenotype, three mutations, including a novel three amino-acid insertion, were identified in one CFC patient and two patients with both NS and CFC phenotypes. These three patients exhibited ectodermal abnormalities such as curly hair, sparse eyebrows and dry skin, and two of them showed mental retardation. Our results suggest that clinical manifestations in patients with SOS1 mutations range from NS to CFC syndrome.

Genome-wide association study identifies susceptibility loci for biliary atresia. *M. Garcia-Barcelo[1], MY. Yeung[2], C. Tang[2], PC. Sham[2,3], S. Cherny[2,3], PK. Tam[1]* 1) Department of Surgery; 2) Department of Psychiatry; 3) Genome Research Centre of the Li Ka Shing Faculty of Medicine of The University of Hong Kong, Hong Kong, China.

Biliary atresia (BA) is characterized by the progressive fibrosclerosing obliteration of the extrahepatic biliary system during the first weeks of life. The incidence of BA ranges from 1 in 5000 to 1 in 18,000 in different populations, the highest being found in Chinese. Despite early diagnosis and prompt surgical intervention, the disease progresses to cirrhosis in many patients. Reported cases of familial BA, together with immunochemistry and gene expression studies on liver tissues, suggest that the immunogenetic vulnerability of the patients to the precipitating factors is likely to play a role in the susceptibility to develop BA. To find susceptibility loci for BA, we conducted genome-wide SNP association study using Affymetrix SNP arrays assaying approximately 500,000 markers on 150 patients and 351 controls. Genotype calling was done using the Birdseed V2 algorithm, yielding an average call rate of 96%. As a preliminary control for stratification, samples were clustered using Multidimensional Scaling (MDS) and the nearest neighbor analysis implemented in PLINK. Samples with evidence of admixture were consequently excluded. To further ensure the accuracy of the study, samples were subjected to biological relationship and cross-examination verification by using the GRR program. SNPs with minor allele frequency less than 5% and call rate lower than 93% were filtered out to produce a panel of around 290K high quality SNPs for genome wide association tests. After stringent quality control, the association tests were conducted on 142 BA patients and 322 controls. Initial data revealed suggestive susceptibility loci on 10q24.2, 5q31.5, and 12q13.3 chromosomal regions.

microRNA regulation of chemoresistance in NSCLC. *A. Pertsemlidis[1], L. Du[1], R. Greer[1], A. Gazdar[1], S. Hammond[2], M. White[1], J. Minna[1]* 1) The University of Texas Southwestern Medical Center, Dallas, TX; 2) University of North Carolina at Chapel Hill School of Medicine, Chapel Hill, NC.

We are trying to determine whether microRNA expression profiles correlate with chemotherapy sensitivity/resistance in lung cancers, whether microRNAs play a functional role in drug sensitivity/resistance of lung cancer cells and whether microRNA expression levels can be manipulated to increase drug sensitivity in cultured lung cancer cells. We have developed a panel of lung cancer and immortalized normal lung epithelial lines for which we have in vitro sensitivity profiles to drugs commonly used in the treatment of lung cancer which can show variations of >1,000 fold, as well as Affymetrix mRNA expression profiles (providing gene expression signatures for sensitivity/resistance). We profiled miRNA expression in a subset of 20 lung cancer lines, and identified miRNAs that that are differentially expressed between cancer and normal and between drug sensitive and resistant NSCLCs. We have identified several miRNAs with expression profiles that are inversely correlated with response to drug treatment in cultured cells. We have transfected chemoresistant NSCLCs with stabilized mimics of these miRNAs and observed a 200- to 300-fold increase in sensitivity to paclitaxel. We also developed a computational method to predict interactions between miRNAs and mRNAs. Based on this method, we predicted that a number of genes potentially related to drug resistance are regulatory targets of miRNAs, including tubulin- and microtubule-associated protein kinases. Combining the predictions with a high-throughput siRNA screen, we identified 20 candidate regulatory targets of microRNAs relevant to chemosensitivity. Several of these predicted targets have been shown to increase cellular sensitivity to paclitaxel when silenced. These data suggest that miRNAs can be both diagnostic of and modulate the response of lung cancers to chemotherapy.

Pre-implantation Genetic Diagnosis (PGD) vs Pre-implantation Genetic Screening (PGS): *A study to determine the acceptance rate of these procedures after genetic counseling during the years 2004 and 2007.* *J. Santolaya-Forgas, C. Jabbour, S. L. McElhinney, M. Hornstein, L. Wilkins-Haug* Ob & Gyn, Brigham & Women's Hospital, Boston, MA.

Objective: PGD and PGS are offered in various centers across the world. Our aim was to determine the rate of acceptance for PGD and PGS after genetic counseling in our center. **Material and Methods:** Our reproductive genetics counseling database was reviewed to obtain information concerning the acceptance rate for pre-implantation genetic testing between the years 2004 and 2007. Patients were divided in 2 groups based on indication for referral. Patients from the PGD group were referred because one member of the couple was a carrier of a disease causing mutation or chromosomal rearrangement. Patients from the PGS group were referred for aneuploidy screening due to advance maternal age, recurrent miscarriages, unsuccessful IVF cycle, previous pregnancy with chromosomal abnormality, or sex selection. All patients were informed about the benefits, limitations and the increased risks for adverse pregnancy outcome associated with assisted reproductive technologies (ART) and pre-implantation genetic testing. Chi-sq test was used to compare the acceptance rate from the PGD and PGS groups. **Results:** Two hundred and seventy one patients received genetic counseling prior to genetic testing during the study period. Overall, 31.3% (85 out of 271) declined pre-implantation genetic testing. Of the 108 patients from the PGD group, 76 (70.4%) accepted pre-implantation genetic testing. Of the 183 patients from the PGS group, 110 (68%) accepted pre-implantation genetic testing (N.S.). **In conclusion:** Our data suggests that during the study period the acceptance rate for PGD and PGS was similar. Studies are know required to determine the impact of recent reports questioning the utility of PGS to improve the rate of take-home baby. Further studies of this type are also recommended to better understand patient's expectations from pre-implantaion genetic testing.

Ethical Aspects of Rare Diseases in Clinical Trials: Gaucher Disease as a Model. *D. Elstein, A. Zimran* Gaucher Clinic, Shaare Zedek Medical Ctr, Jerusalem, Israel.

Albert Einstein has been quoted (1950) to say Morality is not a fixed and stark system. It is a task never finished, something that is always present to guide our judgment and inspire our conduct. Actually Einstein is describing Ethics: whereas Morality is an immutable standard for behavior, Ethics is a resultant of societal mores that accommodates a range of standards based on extant norms. Society may therefore impose constraints that dictate acceptable practice at the expense of some individuals. Meta-ethics is the resultant of this interactive interplay between the individual and society when both sides of the equation cannot be completely satisfied; when this happens society determines what is normative. Thus, in dealing with patients with rare diseases but expensive therapies, some balance must be achieved that serves societal interests because of the issue of limited financial resources. The rare lysosomal disorder, Gaucher disease, albeit the most prevalent storage disorder, serves as a model for these ethical dilemmas because standard enzyme replacement therapy that is both safe and effective is available, yet national health systems must grapple with the ethical equation of the extraordinary expense of this treatment for a relatively small number of citizens with what in most instances is not life-threatening disorder. Recently, new modalities such as substrate reduction therapy, pharmacological chaperones, as well as biosimilar enzyme products, have entered into clinical trials raising new ethical issues such as enrollment in trials when standard therapy is excellent, placebo arms, early-access to investigational drugs, and the critical issues of transparency of data reporting and full disclosure by investigators regarding funding. Finally, one ramification of these ethical concerns is that comparable therapies are being brought to trial for even more rare diseases and for some of these, the clinical efficacy is not as acceptable as for Gaucher disease despite the equally extraordinary cost.

Prevalence of the *MTHFR* C677T polymorphism in patients of Middle Eastern descent. *A. Haghighatgoo[1], Y. Valles-Ayoub[1], C. Saechao[1], S. Esfandiarifard[1], S. Martinez[1], M. Pietruszka[1], D. Darvish[1,2]* 1) HIBM Research Group, Encino, CA; 2) VA Greater Los Angeles (VA-GLA/UCLA), Los Angeles, CA.

The methylenetetrahydrofolate reductase gene (*MTHFR*) 677 C>T polymorphism produces an elevation in plasma homocysteine concentrations. Increased homocysteine levels have been associated with a greater risk for vascular diseases, including cardiovascular disease and ischemic stroke. In this study, we randomly selected and genotyped nucleic acid samples for the C677T allele from our database of Middle Eastern patients, namely Iranian Jews. Results show 52% of patients to be wild-type, 35% to be heterozygous, and 13% to be homozygous for the C677T mutation. This data suggests that the prevalence of the C677T allele in Iranian Jews is slightly higher than what has been reported in populations from varying regions in Iran.

Validation studies on a multiplex quantitative PCR assay for large rearrangements in the *MLH1* and *MSH2* genes for HNPCC. *B. Roa[1], T. Judkins[1], B. Hendrickson[2], K. Bowles[1], K. Eliason[1], J. Schoenberger[1], S. Rajamani[1], J. Trost[1], S. Chen[1], M. Frost[1], G. Rao[1], T. Scholl[2], C. Colvin[1]* 1) Myriad Genetic Laboratories, Inc., Salt Lake City, UT; 2) Genzyme Corporation, Cambridge, MA.

Hereditary non-polyposis colon cancer (HNPCC) is caused by germline mutations in the mismatch repair genes *MLH1*, *MSH2*, *MSH6* and *PMS2*. Approximately 90% of cases are reportedly due to mutations in *MLH1* and *MSH2*, 7-10% to *MSH6*, and <5% to *PMS2*. Clinical testing in our laboratory has shown that 45% of Lynch mutations are due to *MLH1*, 41% from *MSH2*, and 14% from *MSH6*. Mutations in these genes result in an ~80% increased risk of colon cancer and a higher risk for a variety of other cancers. Approximately 5% and 20% of mutations in *MLH1* and *MSH2*, respectively, are large rearrangements that can not be detected by sequencing and require other methods of detection like Southern blot or multiplex ligation-dependent probe amplification (MLPA). We developed and validated the COLARIS Rearrangement Test (CART), that uses quantitative multiplex endpoint PCR analysis and confirmatory testing to detect rearrangements in *MLH1* and *MSH2*. CART uses less DNA and significantly decreases the turnaround time, as compared to Southern blots, while still providing consistent, accurate results. CART consists of 11 multiplexes of 6-12 amplicons each and targets each exon, promoter, and 3UTR of both genes with at least 2 amplicons. Proprietary software normalizes copy numbers for *MLH1* against *MSH2*, and two unlinked control genes. Statistical confidence values are calculated for the presence or absence of duplications or deletions. During a two-phase validation, CART was performed on 130 positive and 541 negative DNA samples in a blinded manner. CART was successful in producing results that were concordant with previous data from routine Southern blots and confirmatory testing. CART will be performed on all patients referred to our laboratory for comprehensive HNPCC testing, along with sequencing of *MLH1* and *MSH2* and/or *MSH6*. The CART assay will facilitate molecular diagnostic testing and will greatly benefit individuals at risk for HNPCC.

Reasons for GJB2/GJB6 testing vary by cultural affiliation: Preliminary data from a collaborative research project on the impact of genetic information on deaf individuals. *C. Palmer[1], P. Boudreault[2], E. Baldwin[1], A. Martinez[1], M. Fox[1], J. Linden[2], R. Trank[1], L. Dutton[1], L. Tullis[1], D. Kovacs[2], L. Perez[2], Y. Kobayashi[2], J. Sinsheimer[1], Y. Sininger[1], W. Grody[1]* 1) UCLA, CA; 2) California State University, Northridge, CA.

Genetic testing for deafness (GTD) is a reality; however, deafness is viewed as a linguistic and cultural trait by many individuals rather than a medical condition. Empirical studies are needed to grasp the ramifications of genetic counseling and testing for deafness. Here we describe a collaborative research model and preliminary data assessing impact of GJB2/GJB6 testing and counseling on deaf adults and the Deaf community using a prospective, longitudinal design. The collaborative model was designed by a hearing, deaf, and hard of hearing research team from diverse disciplines. To include a broad segment of the deaf population, various communication technologies are used and sign language interpreters are provided. Questionnaires (English, Spanish, ASL) assess deaf identity, attitudes, beliefs, testing motivations, knowledge, behaviors, and psychosocial outcomes at four stages. Preliminary data on motivations for GTD at baseline on the first 92 subjects are described. Subjects are 60% female, 86% Caucasian, average age 43y, and 41% ASL-users. Subjects vary in cultural affiliation, with 46%, 15%, 37%, 1% identifying with the Deaf, Hearing, Both, or Neither community, resp. On average, subjects most strongly agreed that learning the cause of deafness and helping research were reasons for GTD, and were neutral or disagreed that GTD would be used to choose a mate. Reasons regarding self, children, family, and deaf community varied by cultural affiliation. These are the first prospectively collected empirical data to examine the relationship between cultural affiliation and GTD. Findings suggest that there is great interest in GTD and cultural factors play a significant role in why deaf adults seek GTD. The results of this culturally sensitive collaborative project provide broader insight into the views of individuals targeted for GTD, and are relevant for deaf individuals, Deaf communities, and genetics services.

A Morpho-Etiological Desription of Congenital Limb Anomalies. *S. M. Tayel[1], F. M. Al Kandary[2], N. A. Al Naqeeb[3], S. Gouda[4], S. A. Al Awadi[4], K. K. Naguib[4]* 1) Anatomy Dept, Genetics Unit, Alexandria Faculty of Medicine, University of Alexandria, Alexandria, Egypt; 2) Faculty of Allied Health Sciences, Kuwait; 3) Neonatology Dep., Adan Hospital, Kuwait; 4) Kuwait Medical Genetics Centre, Maternity Hospital, Kuwait.

BACKGROUND: Limb anomalies rank behind congenital heart disease as the most common birth defects observed in infants. More than 50 classifications for limb anomalies based on morphology and osseous anatomy have been drafted over the past 150 years. The present work aims to provide a concise summary of the most common congenital limb anomalies on a morpho-etiological basis. PATIENTS AND METHODS: In a retrospective study, 70 newborns with anomalies of the upper and/or lower limbs were ascertained through clinical examination, chromosomal analysis, skeletal surveys and other relevant investigations. RESULTS: Fetal causes of limb anomalies represented 55.8% of the cases in the form of 9 cases (12.9%) with chromosomal aberrations (trisomy 13, 18 and 21, duplication 13q and deletion 22q) and 30 cases (42.9%) with single gene disorders. An environmental etiology for limb anomalies was diagnosed in 11 cases (15.7%) as amniotic band disrup¬tion, monozygotic twin with abnormal circulation, vascular disruption (Poland sequence, sirenomelia and general vascular disruption) and an infant with a diabetic mother, Twenty cases (28.5%) had limb anomalies as part of sporadic syndromes of unknown etiology. CONCLUSIONS: The morpho-etiological work-up of limb anomalies adopted in the present study is valuable for detecting the cause of the anomaly and is crucial for its prevention. Prevention can be achieved by proper genetic counseling, which includes recurrence risk estimation and prenatal diagnosis.

Functional analysis of a human *ATPAF2* gene mutation in yeast S.cerevisiae. *S. Seneca[1], A. Meulemans[2], J. Smet[3], R. Van Coster[3], W. Lissens[1], L. De Meirleir[4], I. Liebaers[1], S. Ackerman[5]* 1) Center for Medical Genetics, UZ Brussel, Brussels, Belgium; 2) Laboratory of Pediatrics, ULB, Brussels, Belgium; 3) Department of Pediatrics and Metabolism, UGent, Ghent, Belgium; 4) Department of Pediatric Neurology, UZ Brussel, Brussels, Belgium; 5) Department of Surgery, Biochemistry and Molecular Biology, Wayne State University School of Medicine, Detroit, Michigan, USA.

Aims : Mitochondrial ATP synthase or complex V couples the proton gradient, generated by the respiratory chain, to ATP synthesis. Assembly of this enzyme requires the activities of several accessory proteins. Here, we report on the functional effect of a p.W94R mutation, present in the ATPAF2 gene of an infant with a complex V decreased activity. The ATPAF2 gene is the human (Hu) orthologue of the yeast (y) ATP12 gene. Methods : Multi and single copy plasmid constructs containing the wild type ATPAF2 or ATP12 gene, as well as the Hu mutant p.W94R or the y mutant counterpart p.W103R were generated. These constructs were introduced in an ATP12 respiratory deficient yeast strain. ATP synthase activity of individual yeast transformants was assayed by growth studies on a non-fermentable carbon source and by Blue Native Polyacrylamide Gel Electrophoresis (BN-PAGE) stained for ATP synthase catalytic activity. Plasmid constructs carrying a wt ATP12 or ATPAF2 gene rescue the respiratory defect of a y atp12 mutant strain. Results : Growth on a non-fermentable carbon source was strongly impaired for the Hu W94R mutant, while the growth of the y W103R mutant strain was normal. These results were confirmed by BN-PAGE studies and measurements of the enzymatic complex V activity of the plasmid constructs in yeast cells. Conclusions : Our yeast complementation studies showed clearly that the p.W94R mutant of the huATPAF2p does not confer respiratory competence to an atp12 yeast strain and is most probably the cause of the complex V dysfunction and early death of a 14 months-old patient. In additional studies with atp12fmc1 double mutants, and at 37C, the altered yeast protein was no longer able to rescue the respiratory deficient phenotype of the S.cerevisiae host strain.

Whom Recommended For Screening of The BRCA1 and BRCA2 Mutations? *R. Habibi* Genetics, Royan , Tehran, Tehran, Iran.

Background Only small proportion of breast cancer cases to be due to an inherited predisposition .This condition is increased if there are any susceptibility such as positive family history ,early onset ,involvement before menopause ,bilateral breast cancer and male breast cancer .In this situation , screening for germline mutation in BRCA1 or BRCA2 is important for genetic counseling and cancer risk management . Materials & Methods We studied 104 breast cancer females who were between 25 and 80 years old . Results We found a female case of invasive carcinoma who had 37-year-old ,and positive family history with paternal and maternal involvement .She had involvement in right breast that negative for malignancy and also invasive carcinoma in left breast . Conclusion If the parent /parents are carrier of the mutation , the outcome for the offspring in the generation and family member could be diagnosed and provided the preventive genetic test of the defective gene in the healthy and affected family members . Key words :hereditary ,breast , cancer ,BRCA1 ,BRCA2 ,mutation ,Iranian.

Polymorphisms in immune response and inflammation genes are associated with chronic kidney disease in the U.S. population: data from NHANES III. *R. M. Ned[1,2], A. Yesupriya[1,2], G. Imperatore[3], D. T. Smelser[2,4], R. Moonesinghe[2], M. Chang[2], N. F. Dowling[2], CDC/NCI NHANES III Genomics Working Group* 1) McKing Consulting Corporation; 2) National Office of Public Health Genomics, Centers for Disease Control and Prevention (CDC), Atlanta, GA; 3) National Center for Chronic Disease Prevention and Health Promotion, CDC, Atlanta, GA; 4) ASHG Fellow.

Patients with chronic kidney disease manifest a higher inflammatory state relative to healthy individuals, and therefore, genetic variation in cytokines and other molecules that mediate inflammation or the immune response may play an important role in the pathogenesis of the disease. We studied 34 polymorphisms in 14 genes (*CCR2*, *CRP*, *FCGR2A*, *IL10*, *IL1B*, *IL4*, *IL4R*, *MBL2*, *B9D2/TGFB1*, *NOS2A*, *PPARG*, *TLR4*, *TNF*, and *VDR*) in DNA samples collected from participants in the second phase (1991-1994) of the Third National Health and Nutrition Examination Survey (NHANES III), a population-based, nationally-representative survey of the civilian, non-institutionalized population of the United States. Chronic kidney disease was evaluated in 5,446 participants aged 20 years or older from the three main race/ethnic groups in the U.S. (non-Hispanic white, non-Hispanic black, and Mexican-American). Additive and co-dominant genetic models were tested in both crude and age-sex adjusted analyses. The estimated prevalence of chronic kidney disease was 12.5% in the adult U.S. population, with differences by gender, age, and race/ethnic group. For non-Hispanic whites, variants in *IL10*, *MBL2*, *TLR4*, *TNF*, and *VDR* were associated with chronic kidney disease ($p < 0.05$), as were *CRP*, *MBL2*, *B9D2/TGFB1*, and *VDR* polymorphisms in non-Hispanic blacks and *FCGR2A*, *IL1B*, *B9D2/TGFB1*, and *TNF* variants in Mexican-Americans. This work is the first to investigate the association of genetic polymorphisms with kidney function or chronic kidney disease in a nationally representative sample of the U.S. population.

Evidence for a rheumatoid arthritis protective variant independent of R620W within the PTPN22 locus. *W. R. Wan Taib[1], C. McKinney[1], J. Highton[2], N. Dalbeth[3], L. Stamp[4], P. J. Gow[5], P. B. B. Jones[3], A. A. Harrison[6], S. Steer[7], T. R. Merriman[1]* 1) Department of Biochemistry, University of Otago, Dunedin, New Zealand; 2) Department of Medicine, University of Otago, Dunedin, New Zealand; 3) Department of Medicine, University of Auckland, Auckland, New Zealand; 4) Department of Medicine, University of Otago, Christchurch, New Zealand; 5) Department of Rheumatology, Middlemore Hospital, Auckland, New Zealand; 6) Department of Medicine, University of Otago, Wellington, New Zealand; 7) Department of Rheumatology, Kings College London School of Medicine at Guy's , King's and St. Thomas', London, UK.

Rheumatoid arthritis (RA) is a complex autoimmune disease with a strong genetic contribution to its pathogenesis. The first non-Major Histocompatibility Complex (MHC) gene discovered to be reproducibly associated with RA is the protein tyrosine phosphatase non-receptor 22 (PTPN22) gene in the Caucasian population. The PTPN22 functional variant, R620W, has been associated with RA in many studies in different populations. Carlton et al (Carlton et al, AJHG 77: 567-581, 2005) successfully identified and defined Haplotype 2 that was tagged by R620W and conferred susceptibility effect, whilst haplotypes 5 and 6 conferred protection to RA. We further analysed the two protective haplotypes using three Caucasian case-control cohorts; New Zealand, Wellcome Trust Case Control Consortium (WTCCC) and UK (London). We replicated association with RA using rs3789607 that tags haplotype 5 in 2,717 RA cases and 3,665 controls (OR=0.89, P=0.009). Using rs12144309, which tags haplotype group 6-10 we also replicated this protective effect (OR=0.88, P=0.004) . Within the PTPN22 haplotype block, haplotype 5 and 6-10 coalesced with the major allele of a group of markers within the neighbouring Bfk (B-cell lymphoma 2 family kin) gene. We tested one of the markers, rs12566340, in the New Zealand and WTCCC cohorts for association with RA conditional on rs2476601 (R620W) yielding P=0.13 and P=0.03, respectively. These data provide evidence for a RA protective effect at PTPN22 independent of R620W, mapping outside of PTPN22.

A Systematic Prenatal Diagnosis of a Small Supernumerary Marker Chromosome. *D. Cha[1,2], S. Shim[2], S. Bai[2]* 1) Dept OB/GYN, Kangnam CHA Hosp, Seoul, Korea; 2) Genetic Laboratory, Fertility Center of CHA General Hospital, Pochon CHA University.

A 37-year-old female referred for prenatal chromosome analysis. In previous study of the fetus in other institute, the fetal karyotype was 46,XY,+mar. The marker chromosome was revealed as a de novo from chromosome analyses of parental blood samples. To identify the origin of the marker chromosome, array-CGH was carried out but no informative results were obtained. We re-established the amniotic fluid culture for fetal chromosome analysis. The marker chromosome was a typical small supernumerary marker chromosome apparently originating from an acrocentric chromosome. NOR-staining and FISH analyses with CEP15 and CEP13/21 probes showed the marker chromosome was a biosatellite (NOR-positive in both arms) and CEP 13/21 positive. With careful ultrasound examination, we concluded that the marker chromosome was originated from an inverted duplication of the short arm of chromosome 13 or 21 and the fetal risk with abnormal phenotype was as low as background risk. In this report, we would like to suggest a systematic flow for identification of marker chromosome.

Allele-specific expression due to mutations in the chromatin-associated factor ATRX. *R. J. Gibbons[1], K. M. Lower[1], M. J. Law[1], V. Viprakasit[2], I. Ragoussis[3], A. Morris[3], J. Cross[1], H. Ayyub[1], D. R. Higgs[1]* 1) MRC Molecular Hematology Unit, John Radcliffe Hosp, WIMM, Oxford, OX3 9DS, UK; 2) Division of Medical Genetics and Molecular Medicine, Department of Medicine, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand; 3) The Wellcome Trust Centre for Human Genetics, Roosevelt Drive, Oxford, OX3 7BN, UK.

Genetically determined patterns of gene expression underlie much of the phenotypic variation found in both normal individuals and those with inherited and acquired diseases. Whole genome association studies have shown that levels of expression for many genes are associated with polymorphic *cis* and *trans* acting elements called expression quantitative trait loci (eQTLs) although the identities of the true regulatory polymorphisms are largely unknown. As might be expected *cis* eQTLs are associated with unequal allelic expression. The X linked gene *ATRX* encodes a SWI/SNF-like chromatin associated factor and transacting mutations give rise to ATR-X syndrome. The condition is associated with variable levels of thalassemia due to downregulation of globin gene expression. The severity of thalassemia may be treated as a quantitative trait.

Here we show that mutations in ATRX (acting as a *trans* eQTL) may downregulate globin and a closely linked, newly identified, target gene (called *NME4*) in an allele-specific manner, producing allele-specific differences in gene expression. Often but not invariably, the downregulation is associated with CpG island methylation. In each case there is compelling evidence that the associated *cis* eQTLs are G-rich variable number tandem repeats (VNTRs). Not only are these VNTRs the sites of peak ATRX binding but in cases which are heterozygous for the VNTR, the larger allele of the VNTR at *NME4* is always preferentially downregulated and methylated (n=55). These observations demonstrate that, contrary to expectations, *trans* eQTLs may be restricted by the regulatory haplotypes containing their target genes. This adds important new insights to the nature of interactions between *trans* and *cis* eQTLs, the phenotypic variability to which these give rise and the function of the ATRX protein.

Confounded by association: A general principle for the identification of causal genetic effects in association studies. *C. Lange* Dept Biostatistics, Harvard Sch Public Health, Boston, MA.

In genetic association studies, different complex phenotypes are often associated with the same marker. Such associations can be indicative of common genetic causes, of indirect genetic effects via one of these phenotypes, or can be solely attributable to non-genetic/environmental links between the traits. In the analysis, the multiple associations disguise the causative genetic effect and aggravate its identification. To identify the phenotypes with the inducing genetic association, statistical methodology is needed to distinguish between the different origins of the associations. We propose a simple, general adjustment principle that can be incorporated into virtually all standard genetic association tests which are then able to infer whether a SNP has a direct biological influence on a given trait other than through the SNPs influence on another correlated phenotype. The proposed adjustment is straightforward to compute and is robust against population admixture. It is particularly relevant for genome-wide association studies, pathway analysis, and association studies with an integrative genomic component. Using simulation studies, we show that, in the presence of a non-marker related link between phenotypes, standard association tests without the proposed adjustment can be biased. In contrast to that, the proposed methodology remains unbiased and has sufficient power to infer causal genetic effects for realistic sample sizes. Its achieved power levels are identical to those of standard methodology, making the adjustment principle universally applicable in genetic association studies. The principle is illustrated by an application to three genome-wide association studies.

A novel multi-loci Bayesian approach to genome-wide association studies with family-based designs. *M. Naylor, C. Paciorek, C. Lange* Dept of Biostatistics, Harvard University, Boston, MA.

For genome-wide association studies with family-based designs, we propose a multi-loci Bayesian approach. We show that standard TDT/FBAT statistics can naturally be implemented in a Bayesian framework and that informative priors can be constructed for each marker based on the actual data. In contrast to Bayesian approaches that have recently been proposed for genome-wide association studies, our approach does not require prior assumptions about the genetic effect size; this makes the proposed method entirely data-driven. For the construction of the priors, the evidence for association for a single marker is obtained at the population-level by estimating the posterior distribution of the genetic effect sizes in the conditional mean model. Since such genetic effect size estimates are statistically independent of the effect size estimation within the families, the actual data set can inform the construction of the priors without any statistical penalty. The power of the approach is assessed through simulation studies.

IL23R in the Swedish, Finnish, Hungarian and Italian populations: Association with inflammatory bowel disease and psoriasis, and linkage to celiac disease. *E. Einarsdottir[1], L. L. E. Koskinen[1], E. Dukes[1], K. Kainu[2], F. Ziberna[3], I. R. Korponay-Szabo[4], K. Kurppa[5], K. Kaukinen[5], R. Ádány[4], T. Not[3], A. Ventura[3], R. Löfberg[6], L. Torkvist[6], F. Bresso[6], J. Halfvarson[7], M. Mäki[5], U. Saarialho-Kere[2], J. Kere[1,6], M. DAmato[6], P. Saavalainen[1]* 1) University of Helsinki, Helsinki, Finland; 2) Helsinki University Hospital, Helsinki, Finland; 3) University of Trieste, Trieste, Italy; 4) University of Debrecen, Debrecen, Hungary; 5) Tampere University Hospital, Tampere, Finland; 6) Karolinska Institutet, Huddinge, Sweden; 7) Örebro University Hospital, Örebro, Sweden.

Background: The recently reported association of interleukin 23 receptor (IL23R) with inflammatory bowel disease (IBD) has been confirmed in several populations. The IL23R gene also associates with psoriasis and ankylosing spondylitis, making it an interesting candidate for many immunological diseases. **Materials and methods:** We studied association of single-nucleotide variants in IL23R with IBD in Swedish patients with Crohns disease (CD) and ulcerative colitis (UC), the two major forms of IBD. The same variants were studied in Finnish psoriasis patients and in Finnish, Hungarian and Italian patients with celiac disease. In total, 2924 cases and 1030 controls were studied. **Results:** We replicated the association of IL23R with IBD in Swedish IBD patients, and found linkage and association of IL23R with psoriasis in the Finnish population. IL23R was linked to celiac disease in Finnish, but not Hungarian families. No significant association was seen with IL23R in the celiac datasets. Western blot analysis suggests that the genetic variants analyzed in this study do not directly affect levels of IL23R protein. **Conclusions:** Our study further supports the involvement of IL23R in IBD in Swedish patients, and the linkage and association of IL23R with psoriasis in the Finnish population. We also found linkage of IL23R to celiac disease in the Finnish population, and postulate that other variants in IL23R or in a nearby gene may be associated with celiac disease.

Association study of *IL18RAP* in three European populations with celiac disease. *L. L. E. Koskinen[1], E. Einarsdottir[1], E. Dukes[1], G. A. R. Heap[2], P. Dubois[2], I. R. Korponay-Szabo[3,4], K. Kaukinen[5], K. Kurppa[5], F. Ziberna[6], S. Vatta[6], T. Not[6], A. Ventura[6], R. Ádány[4], Z. Pocsai[4], G. Széles[4], M. Mäki[5], J. Kere[1,7], C. Wijmenga[8], D. A. van Heel[2], P. Saavalainen[1]* 1) University of Helsinki, Helsinki, Finland; 2) Queen Mary University of London, London, UK; 3) Heim Pal Childrens Hospital, Budapest, Hungary; 4) University of Debrecen, Debrecen, Hungary; 5) University of Tampere and Tampere University Hospital, Tampere, Finland; 6) University of Trieste, Trieste, Italy; 7) Karolinska Institutet, Huddinge, Sweden; 8) University Medical Center Groningen and University of Groningen, Groningen, Netherlands.

Background: Celiac disease is caused by dietary gluten, which triggers chronic inflammation of the small intestine in genetically predisposed individuals. Recently, a novel risk locus on chromosome 2q11-q12, harboring *IL18RAP*, was identified for celiac disease. IL18 has been shown to play an important role in Th1 activity in celiac disease, making IL18RAP a highly interesting candidate gene.

Materials and methods: In this study, two previously indicated risk variants at the *IL18RAP* locus were tested for association in 1210 cases with celiac disease and 1033 controls from the Finnish, Hungarian, and Italian populations. The protein expression of IL18RAP was compared between risk allele carriers and non-carriers.

Results and conclusions: We confirmed genetic association, and in addition found a dose effect of *IL18RAP* genotypes with celiac disease in the Hungarian population. The GA haplotype of the two markers showed the strongest association (P=0.0001, OR=1.5) in the Hungarian, as well as in the combined material (P=0.0002, OR=1.3). Putative isoforms of IL18RAP were detected and cells with risk background showed higher levels of protein expression than cells with protective background. Our study supports IL18RAP as a novel predisposing gene for celiac disease and highlights the need for further functional studies on this relatively unknown gene in celiac disease pathogenesis.

Matrilin-3 - an essential protein in the normal development of cartilage and bones. Progress in structure determination. *V. Adir[1], A. Shahar[2], N. Adir[2], Z. U. Borochowitz[1]* 1) The Simon Winter Institute for Human Genetics, Bnai-Zion Medical Center, Rappaport Faculty of Medicine,Technion, Haifa, Israel; 2) Schulich Faculty of Chemistry, Technion, Haifa, Israel.

Matrilin-3 (MATN3) belongs to the matrilin family of extracellular matrix proteins and is primarily expressed in cartilage. Mutations in the gene encoding human MATN3 lead to skeletal disorders, such as Spondylo-Epi-Metaphyseal dysplasias (SMED). A novel form of SMED that was report by us, is caused by a homozygote substitution 973TA in the MATN3 gene. The defect is characterized by short stature and early-onset osteoarthritis. The majority of disease-causing mutations are located within the predicted -sheet of the single von Willebrand Factor A (vWFA) domain of MATN3, suggesting that they disrupt the structure and/or function of this important domain. Structural insights into this protein family's function have not yet been achieved. In order to obtain such insights, the gene encoding for the MATN3 from Mus musculus was cloned and overexpressed in E.coli. In addition, the vWFA domain was also cloned into an expression vector. In both cases the protein was expressed as insoluble inclusion bodies which were recovered by the use of 1% SDS and urea. We are currently working on modifications of the purification process of the MATN3 protein and the vWFA domain in order to obtain large amounts of pure and soluble protein. This will then be used for crystallization and structure determination.

Association in the 15q13-14 Schizophrenia Linkage Region. *S. H. Stephens[1], A. L. Franks[2], R. Berger[2], S. Leonard[2]*
1) Institute for Behavioral Genetics, University of Colorado, Boulder, CO; 2) Dept of Psychiatry, University of Colorado Health Sciences Center, Aurora, CO.

Schizophrenia has been linked to the 15q13-14 locus in multiple independent studies and across ethnicities. The 7 neuronal nicotinic acetylcholine receptor subunit gene (*CHRNA7*) maps to this chromosomal region and was selected as the best candidate gene for an endophenotype of schizophrenia, the P50 sensory processing deficit, based on genetic linkage to the locus and human and animal studies. Mutation screening of *CHRNA7* gene identified functional polymorphisms in the upstream regulatory region. However, the mutations isolated thus far do not account for linkage to the 15q13-14 locus. The current study addresses this issue by fine mapping genes in the 15q13-14 linkage peak region to determine if genetic variance in the *CHRNA7* gene accounts for the linkage to schizophrenia. Family-based and case-control association was performed on samples from 120 families as well as 468 schizophrenic patients and 144 controls. 1217 Single Nucleotide Polymorphism (SNP) markers and three informative microsatellite markers were utilized. Association with the outcomes of schizophrenia, smoking, smoking in schizophrenia, and the P50 deficit in controls were performed. Three genes were associated with schizophrenia in both ethnic populations, after correction for multiple testing: *TRPM1*, *KLF13*, and *RYR3*. One SNP in *CHRNA7* (rs8028396) was significant for an association with smoking and smoking in schizophrenia in Caucasians. A SNP in *RYR3* (rs8035184) was associated with smoking in African-Americans. Association studies with the P50 deficit in controls found significant P-values for SNPs in genes *CHRNA7*, *RYR3*, *SGNE1*, and *AVEN*. These studies continue to support *CHRNA7* as a candidate gene for schizophrenia and introduce three new genes in the 15q13-14 linkage region as additional candidate genes for the disorder.

Nav1.7 channel mutations cause two different clinical phenotypes. *T. Fischer[1,2,3], E. Gilmore[1,2,3], M. Estacion[1,2,3], B. W. Jarecki[4], L. Tyrrell[1,2,3], S. Taylor[5], M. Melanson[5], M. Lawden[4], T. R. Cummins[6], S. D. Dib-Hajj[1,2,3], S. G. Waxman[1,2,3]* 1) Dept Neurology, Yale Univ, New Haven, CT; 2) Center for Neuroscience & Regeneration Research, Yale Univ, New Haven, CT; 3) Rehabilitation Research Center, VA CT Healthcare System, West Haven, CT; 4) Dept Neurology, Leicester General Hospital, Leicester UK; 5) Dept Neurology, Kingston General Hospital, Ontario, Canada; 6) Dept Pharmacology & Toxicology, Stark Neurosciences Institute, Indiana Univ School of Medicine, Indianapolis, IN.

Human and animal studies have shown that the Nav1.7 sodium channels play a major role in inflammatory and neuropathic pain. Early-onset inherited erythromelalgia (IEM) is the first known human pain syndrome to be examined at a molecular level, and has been linked to gain-of-function mutations in the Nav1.7 sodium channel. A different set of mutations in Nav1.7 underlie another Nav1.7-related painful neuropathy, paroxysmal extreme pain disorder (PEPD). In contrast to IEM, PEPD has been effectively treated with the sodium channel blocker carbamazepine. We now report a novel mutation (V400M) in a three-generation Canadian family with early-onset IEM located within domain I/S6 in Nav1.7. Interestingly, the pain symptoms in these patients respond favorably to treatment with carbamazepine. Furthermore, we have identified a mutation (M1627K) in a two-generation English family with PEPD located within domain IV/S4-5. The proband and her sister from this family also respond favorably to treatment with carbamazepine. The biophysical properties of the mutant channels were evaluated using whole-cell patch clamp studies in stably-transfected HEK293 cells. Boltzman fits of activation and fast-inactivation curves show a hyperpolarizing shift in activation and a depolarizing shift in fast-inactivation for V400M channels as compared to wild-type channels. Electrophysiologic studies on cells containing the M1627K mutation show a shift in voltage-dependence of fast-inactivation and inactivation from the open state with slower kinetics. Using current-clamp methodology, both mutations have been shown to render DRG neurons hyperexcitable, yet they underlie different clinical phenotypes.

Autism and Cognitive-Behavioral Features Of Children With Subtelomeric Deletions. *G. Fisch[1], J. Carey[2], J. Youngblom[3], R. Simensen[4], A. Battaglia[5]* 1) NYU, New York, NY; 2) University of Utah, Salt Lake City, UT; 3) California State University, Turlock, CA; 4) Greenwood Genetics Center, Greenwood, SC; 5) University of Pisa, Pisa, Italy.

Cognitive-behavioral features of children with subtelomeric deletions have not been systematically evaluated. The aim of our study was to assess cognitive-behavioral features of children with 4 different subtelomeric deletions, using a comprehensive neuropsychological battery. Our assessment battery consisted of 5 standardized measures of cognitive ability, adaptive behavior, emotionality and temperament, attentiveness/ hyperactivity, and autism. We examined 29 children, ages 4-17 years, with del2q37 [n=7], del8p23 [n=7], del11q25 n=3], or 4p16 [n=12], from 9 sites in the US and Europe. We found 10/29 (34%) of our sample who had CARS scores 30. That is, more than a third of the sample could be diagnosed as autistic. In addition, each disorder was associated with a distinct cognitive-behavioral profile. Children with de11q25 had significantly higher cognitive abilities, while those with del4p16 were significantly lower. Adaptive behavior was significantly higher among children with 11q25. Cognitive ability and adaptive behavior profiles were also statistically significantly different among the groups. Attention deficits and hyperactivity were also noted in 18/29 [62%] of children assessed.

HRAS mutations and 11p allelic imbalance in a rare case of agminated Spitz nevus in a giant nevus spilus. *K. Sol-Church[1], D. L. Stabley[1], S. Catalano[1], J. Holbrook[1], J. B. Lee[2], K. Conard[3], P. Hyde[2], D. Shurman[2]* 1) Dept Biomedical Research, Alfred I duPont Hosp Children, Wilmington, DE; 2) Department of Dermatology and Dermatopathology, Thomas Jefferson University, Philadelphia, PA; 3) Dept Pathology, Alfred I duPont Hosp Children, Wilmington, DE.

Background: Differentiating a Spitz nevus from a Spitzoid melanoma is often a challenging task faced by dermatopathologists. While the dysplastic nevus theory supports the notion that a Spitz nevus can progress to a melanoma, there is molecular evidence that refutes this possibility. Namely, Spitz nevi can carry unique mutations found in the mitogen-activated protein kinase pathway that are not found in melanomas. Thus it would be extremely unlikely that a Spitz nevus could lose a unique mutation to transform into melanoma. **Methods**: In this report, the cytogenetics and detailed molecular analysis was performed on a rare and fascinating case of agminated Spitz nevus in a giant nevus spilus. We used DNA sequencing as well as STR-PCR and real time PCR for genotyping and copy number evaluation. **Findings**: The analysis revealed two *HRAS* mutations located on the maternally inherited allele. The *HRAS* mutations observed in the patients Spitz nevi, result in A11S and G13R amino acid substitutions in the ras protein. *HRAS* copy number increase, as well as allelic imbalance at 11p with gain of the maternal allele, was also observed in tissue containing the mutated gene. **Interpretations**: Differential gene dosage effect of important imprinted genes located on the short arm of chromosome 11 may counteract the effects of *HRAS* activation in Spitz nevi, and prevent malignant transformation and may explain why no case of conversion of multiple Spitz nevi to malignant melanoma has ever been reported.

***De novo* mutations in the gene encoding STXBP1 (MUNC18-1) cause early infantile epileptic encephalopathy with suppression-burst.** *H. Saitsu[1], M. Kato[2], T. Mizuguchi[1], K. Hamada[3], H. Osaka[4], J. Tohyama[5], K. Uruno[6], S. Kumada[7], K. Nishiyama[1], A. Nishimura[1], I. Okada[1], Y. Yoshimura[1], S. Hirai[8], T. Kumada[9], K. Hayasaka[2], A. Fukuda[9], K. Ogata[3], N. Matsumoto[1]* 1) Dept Human Gen, Grad Sch Med, Yokohama City Univ, Yokohama, Japan; 2) Dept Pediatrics, Yamagata Univ Sch Medicine, Yamagata, Japan; 3) Dept Biochemistry, Grad Sch Med, Yokohama City Univ, Yokohama, Japan; 4) Division of Neurology, Clin Res Ins, Kanagawa Children's Medical Center, Yokohama, Japan; 5) Dept Pediatrics, Epilepsy Center, Nishi-Niigata Chuo National Hospital, Niigata, Japan; 6) Epilepsy Center, Yamagata National Hospital, Yamagata, Japan; 7) Dept Neuropediatrics, Tokyo Metropolitan Neurological Hospital, Fuchu, Japan; 8) Dept Mol Biol, Grad Sch Med, Yokohama City Univ, Yokohama, Japan; 9) Dept Physiology, Hamamatsu Univ Sch Med, Handayama, Japan.

Early infantile epileptic encephalopathy with suppression-burst (EIEE), also known as Ohtahara syndrome, is one of the most severe and earliest forms of epilepsy. Using array-based comparative genomic hybridization, we found a *de novo* 2.0-Mb microdeletion at 9q33.3-q34.11 in a female EIEE patient. Mutation analysis of candidate genes mapped to the deletion revealed that four unrelated EIEE patients had heterozygous missense mutations in *syntaxin binding protein 1* (*STXBP1*). STXBP1 (also known as MUNC18-1) is an evolutionally conserved neuronal Sec1/Munc-18 protein, which plays an essential role for synaptic vesicle release in multiple species. Transient expression in Neuroblastoma 2A cells showed that all mutant STXBP1 proteins tend to aggregate. Circular dichroism melting experiments revealed that a mutant protein was significantly thermolabile compared to the wild type. Furthermore, binding of the mutant protein to syntaxin was impaired. These findings suggest that haploinsufficiency of STXBP1 causes EIEE.

Binding sites for ETS family of transcription factors dominate the promoter regions of differentially expressed genes in abdominal aortic aneurysms. *J. Nischan[1], Z. Gatalica[2], M. Curtis[2], G. M. Lenk[1], G. Tromp[1], H. Kuivaniemi[1]*
1) Center for Molecular Medicine and Genetics, Wayne State University, Detroit, MI; 2) Department of Pathology, Creighton University School of Medicine, Omaha, NE.

Abdominal aortic aneurysm (AAA) is a life threatening disease affecting about 10% of the population over the age of 65 and causing over 15,000 deaths per year in the US. AAAs are characterized by local chronic inflammation of the aortic wall, decreased numbers of smooth muscle cells in the aortic media layer and fragmentation of the extracellular matrix. Our previous study identified 3,274 distinct differentially expressed genes in AAA compared to age, sex and ethnicity matched non-aneurysmal aortas. Using transcriptional genomics we investigated whether there were common transcriptional regulatory elements in the promoter regions of the differentially expressed genes. We used Whole Genome rVISTA to analyze the differentially expressed gene sets with increased and decreased expression to determine the transcription factor binding sites (TFBSs) overrepresented in the 5 kb promoter regions of these 3,274 genes. There were 144 transcription factors (TFs) whose TFBSs were overrepresented in the subset with decreased expression as compared to the entire genome. In contrast, there were only 13 overrepresented TFs in the set with increased expression. TRANSFAC classification showed 8/13 enriched TFs in the gene set with increased expression belonged to the ETS-type family of TFs. Additionally, NFKB and its heterodimeric subunits p65 and p50 showed enrichment in this subset. Both NFKB and many members of the ETS family of TFs have been previously implicated in vascular inflammation and remodeling and have been targets for investigation for their roles in AAA. By immunohistochemistry, 7 of 8 TFs were present in the adventitia and media of aneurysmal tissue. This is consistent with our microarray study, which showed that the same 7 TFs were expressed in aneurysmal tissue. Our results provide additional information on the role of the ETS family in the pathogenesis of AAA offering potential therapeutic targets for the management of this deadly disease.

MicroRNA Profiling of Human Lens Cell Lines HLE-B3 and SRA01/04. *L. Tian, K. Huang, D. Stambolian*
Department of Ophthalmology, University of Pennsylvania, Philadelphia, PA 19104, PA.

Many miRNAs are expressed in a developmentally regulated and tissue-specific manner making them crucial for tissue development in structures such as the eye. Most of the miRNA target function studies for the eye will need to be performed in ocular tissue culture cells. To date, miRNA profiles for the standard cell lines representative of the eye are nonexistent. We used miRNA microarrays to determine the relative level of miRNA expression in two human lens epithelial cell lines, HLE-B3 and SRA01/04. The Limma data analysis package was used to perform global loess normalization, and estimate fold changes. Multiple test adjusted p-values were obtained by the Benjamini and Hochbergs method with a false discovery rate below the threshold value of 1%. Four microRNAs showed more than 2-fold expression differences between SRA01/04 and HLE-B3. These miRNAs included mir-184, mir-31, mir-100 and mir-222. Differences in miRNA profiling were significant between the human cell lines and mouse lens. Comparison of the miRNA profile of either cell line to mouse lens miRNA demonstrated 70 miRNAs that had >2-fold relative expression change. A molecular function annotation was performed using DAVID to further characterize the pathways influenced by the 70 differentially expressed miRNAs. Grouping of target genes into pathways were significant for adhesion, cell death, cell cycle, post-translational protein modifications, transport and cell communication.

Why dont deaf people come for genetic counselling? Quantitative and qualitative findings from a UK study. *A. Middleton[1], S. Emery[2], G. H. Turner[2], A. Clarke[1], S. Sarangi[3], M. Bitner-Glindzicz[4], M. Richards[5], D. Stephens[6]* 1) Institute of Medical Genetics, Cardiff University, Cardiff, South Glamorgan, United Kingdom; 2) Department of Languages & Intercultural Studies, School of Management & Languages, Heriot-Watt University, Edinburgh, United Kingdom; 3) Health Communication Research Centre, School of English, Communication and Philosophy, Cardiff University, United Kingdom; 4) Clinical & Molecular Genetics, Institute of Child Health, London, United Kingdom; 5) Centre for Family Research, Cambridge University, United Kingdom; 6) Welsh Hearing Institute, School of Medicine, Cardiff University, United Kingdom.

Despite genetic deafness affecting more than 1 in 2000 people, deaf adults very rarely access genetic counselling in the UK, neither to discuss deafness nor other conditions that may be running through their family, e.g. cancer. Each genetics centre in the UK has several thousand deaf sign language users within their catchment area; however, referrals are likely to be received for fewer than 5 deaf adults per year. There may be many complex reasons behind this - e.g. lack of information, assumptions about inheritance or fears about being told not to have children. This project aims to gather the views of deaf and hard of hearing people, looking specifically at the above issues. 30 interviews have been conducted in sign language and qualitative analysis performed. A structured postal questionnaire has been completed by more than 1000 participants. The results indicate that most deaf people dont know how to get genetic counselling. Of those that are interested in having genetic counselling, most want to know about deafness rather than other conditions. Of those not interested in having genetic counselling, most assume that the deafness in their family is not inherited, even if they have a strong family history. Lack of information, assumptions about inheritance, negative attitudes towards genetics and communication difficulties all appear to play a part in preventing access to services. Once more is understood about these issues then steps can be taken to address them.

Timing of Imiglucerase Initiation and Other Risk Factors for Avascular Necrosis in Type 1 Gaucher Disease. *N. Weinreb[1], P. Deegan[2], A. Vellodi[3], J. A. Cole[4], M. Yeh[4], P. K. Mistry[5]* 1) Univ Research Foundation, Hollywood, FL; 2) Addenbrooke's Hospital, Cambridge, UK; 3) Great Ormond Street Children's Hospital NHS Trust, London, UK; 4) Genzyme Corporation, Cambridge, MA, USA; 5) Yale University School of Medicine, New Haven, CT.

OBJECTIVE: Treatment of type 1 GD (GD1) is initiated at varying intervals following diagnosis. This study assessed the effect of elapsed time from diagnosis to initiation of enzyme therapy with alglucerase or imiglucerase on the subsequent risk of developing avascular necrosis (AVN), the principal bone manifestation of GD1. The secondary aim was to identify other determinants of AVN. METHODS: All alglucerase/imiglucerase-treated patients with GD1 enrolled in the ICGG Gaucher Registry without documented AVN prior to initiation of therapy were included. Incidence rate for the first occurrence of AVN after starting therapy was calculated according to time intervals from diagnosis to initiation of therapy. Other risk factors investigated included spleen status, GBA genotype, age at therapy initiation and enzyme dose. RESULTS: 2,700 patients met the inclusion criteria. Among GD1 patients who began alglucerase/imiglucerase 2 or more years after diagnosis, the incidence rate of AVN was 16.6 per 1,000 person-years. Patients with an interval to start of treatment less than 2 years had a lower rate of AVN (incidence rate 8.1 per 1,000 person-years; adjusted incidence rate ratio <2 years vs. >2 years 0.58, 95% confidence interval 0.37 - 0.91). Patients with antecedent splenectomy (total or partial) had a higher incidence rate of AVN, regardless of the timing of treatment initiation (incidence rate 26.8 per 1,000 person-years; adjusted incidence rate ratio vs. non-splenectomized patients 2.35, 95% confidence interval 1.73-3.18) CONCLUSION: With an interval of more than 2 years between GD1 diagnosis and initiation of therapy, patients have increased risk of post-treatment osteonecrosis.

Genome-wide screen of late-onset Alzheimer's disease identifies TRPC4AP in two large pedigrees. *S. Poduslo*[1,2], *R. Huang*[1], *J. Huang*[1], *S. Smith*[1] 1) Inst Molec Medicine & Genetics, Medical Col Georgia, Augusta, GA; 2) VA Medical Center.

Alzheimer's disease is a complex disease with profound cognitive decline. Two extended pedigrees with many siblings affected with late-onset Alzheimer's disease were used in a genome-wide screen, using SNP microarrays, containing 500,000 SNPs. Significant SNPs were identified in one gene, after Bonferroni correction. Additional SNPs were analyzed in the extended families, with unaffected spouses as controls. All of the affected siblings had the same haplotype for ten SNPs in the gene, while the unaffected spouse controls had a different haplotype. TRPC4AP (transient receptor potential cation channel, subfamily C member 4 associated protein) on chromosome 20q11.22 is part of the TRP family which functions in regulating calcium. The gene has 19 exons and is 97,000 bp. The gene is being resequenced to identify the mutation. Supported by funds from a VA Merit award and MCG startup funds.

Defects in cell polarity underlie renal cystic disease in tuberous sclerosis. *C. S. Bonnet[1], C. von Ruhland[2], R. Harris[1], R. Sandford[3], J. P. Cheadle[1]* 1) Institute of Medical Genetics, Cardiff University, Cardiff, CF14 4XN, United Kingdom; 2) Medical Microscopy Sciences, Cardiff University, Cardiff, CF14 4XN, United Kingdom; 3) Department of Medical Genetics, University of Cambridge, Cambridge, CB2 0XY, United Kingdom.

Clinical trials are underway for the treatment of tuberous sclerosis (TSC)-associated tumours using mTOR inhibitors. Here, we show that many of the earliest renal lesions from *Tsc1*$^{+/-}$ and *Tsc2*$^{+/-}$ mice do not exhibit mTOR activation, suggesting that pharmacological targeting of an alternate pathway may be necessary to prevent tumour formation. Defects in the structure or function of primary cilia underlie numerous disorders associated with cystic kidneys such as autosomal dominant polycystic kidney disease (ADPKD). Given that patients with TSC often develop renal cysts, we tested for a functional interaction between the *TSC1* and *TSC2* gene products (hamartin and tuberin) and the *ADPKD1* gene product (polycystin-1), by crossing *Tsc1*$^{+/-}$ and *Tsc2*$^{+/-}$ mice with *Pkd1*$^{+/-}$ mice. We found that compound heterozygous mice had significantly more renal cysts as compared to single heterozygotes and that hamartin, tuberin and polycystin-1 helped maintain the length of primary cilia in pre-cystic renal tubule cells. Consistent with the observation that primary cilia modulate the planar cell polarity (PCP) pathway, we found that many dividing cells from *Tsc1*$^{+/-}$, *Tsc2*$^{+/-}$ and *Pkd1*$^{+/-}$ mice were highly misorientated along the tubule axis. We therefore propose that defects in cell polarity underlie both ADPKD and TSC-associated renal cystic disease and targeting of this pathway may be of key therapeutic benefit.

A case of Down syndrome with "mirror image" duplication of chromosome 21. *T. H. T. Minh[1], N. V. Nihan[1], N. M. Lindor[2], R. G. Meyer[3], R. Rai[3], G. V. N. Velagaleti[3]* 1) Dept. Medical Genetics, Hue College of Medicine & Pharmacy, Hue City, Viet Nam; 2) Dept. Medical Genetics, Mayo Clinic, Rochester, MN; 3) Dept. Laboratory Medicine & Pathology, Mayo Clinic, Rochester, MN.

A 12-year-old girl was referred for cytogenetic analysis because of moderate mental retardation and features typical of Down syndrome (DS). Family history is significant as two of her siblings with apparent DS died during infancy. The mother has two spontaneous abortions. The mother was 22 years old and the father was 34 at the time of proband's birth. Chromosome analysis showed a karyotype 46,XX,idic(21)(q22.3) in all the 100 metaphases analyzed. AgNOR staining on the abnormal chromosome showed satellites at both ends suggesting it is dicentric. Parental chromosome analyses from peripheral blood showed normal karyotypes. FISH analysis with chromosome 13/21 alpha satellite probes confirmed the presence of two centromeres on idic(21). FISH analysis with LSI 21, AML1 and subtelomere probes also showed duplication of signals, although the telomere signals showed overlapping signals making precise determination of telomere status difficult. FISH using LSI 21 probe on parents blood lymphocytes did not show mosaicism for a balanced rearrangement. Although trisomy 21 is one of the most common chromosome abnormality, DS due to mirror image duplication of chromosome 21 is extremely rare with fewer than 10 cases reported. The mechanism of these mirror image duplications is not clearly elucidated, but translocation between sister chromatids or intrachromosomal interchange has been suggested as one mechanism. The majority of the mirror image DS cases are reported as sporadic mutational events with no significant family history, unlike our case. The family history of 3 affected siblings and 2 spontaneous abortions and normal chromosome results from peripheral blood in parents suggests a strong possibility that there might be gonadal mosaicism in our family. Further FISH studies are in progress to analyze the sperm for idic(21) in the father. To the best of our knowledge, this is the first case of mirror image duplication of chromosome 21 leading to DS with a strong possibility of gonadal mosaicism.

The Genetic Information Nondiscrimination Act of 2008: Minimal Protections but Maximum Publicity. *J. Wagner* Dept Anthropology, Penn State Univ, University Park, PA.

For over a decade Congress has tinkered with legislation to prohibit genetic discrimination. Accumulating suspicions of misuse in employment and insurance contexts following the realization of the Human Genome Project and the growing popularity of genetic discoveries in the news sparked Congress into action. On May 21, 2008, the Genetic Information Nondiscrimination Act of 2008, known colloquially as GINA, was signed into law. Yet the practical impact of GINA is far from certain, as the bill neglects as much as it addresses. Geneticists must not only be aware of the state of the laws but also be aware of how laws are shaped and refined over time. This research analyzes the legislative history of GINA, its legislative precursors, and the framework within which it was constructed. The text and scope of genetic nondiscrimination laws at the state-level are analyzed in relation to GINA and the medias reporting of GINA is surveyed. GINA amends Employee Retirement Income Security Act, Public Health Service Act, and Internal Revenue Code. GINA neglects the use of genetic predispositions to limit damages or deny causality in medical tort cases. While defining genetic information broadly to include family history, GINAs applicability is narrow, giving a potential false sense of security. For example, while insurers are forbidden from increasing premiums for someone found to carry the BRCA1 mutation, the insurers are still permitted to use their business judgment to determine whether a double mastectomy is elective or medically necessary, subject only to an abuse of discretion review. GINA prohibits employment discrimination on the basis of genetic information yet intentionally fails to provide a cause of action to such a victim: The efficacy of such an empty threat is doubtful. Moreover, GINA does not apply to employers who have less than 15 employees. While the passage of GINA and the medias advertisements of its protections may alleviate the publics concerns for misuse of genetic information, GINA opens a new host of challenges and dangers to those seeking or receiving genetic testing.

***Sall1*, *Sall2*, and *Sall4* are required for neural tube closure in mice.** *J. Kohlhase[1], J. Böhm[2], A. Buck[3], W. Borozdin[1,2], A. U. Mannan[3], U. Matysiak-Scholze[2], I. Adham[3], W. Schulz-Schaeffer[4], T. Floss[5], W. Wurst[5], F. Barrionuevo[2]* 1) Dept Human Genetics, Ctr Human Genetics Freiburg, Freiburg, Germany; 2) Inst Human Genetics, University of Freiburg, Freiburg, Germany; 3) Inst Human Genetics, University of Goettingen, Goettingen, Germany; 4) Dept Neuropathology, University Clinic Goettingen, Goettingen, Germany; 5) GSF National Research Center for Environment and Health, Institute of Developmental Genetics, Neuherberg, Germany.

Four homologs to the Drosophila homeotic gene spalt (sal) exist in humans and mice (*SALL1-4*/*Sall1-4*). Mutations in *SALL1* and *SALL4* result in the autosomal-dominant developmental disorders Townes-Brocks and Okihiro syndrome, respectively. In contrast, to date, no human disease has been associated with *SALL2*, and *Sall2*-deficient mice showed no apparent abnormal phenotype. We have generated mice deficient in *Sall2*, and in contrast to previous reports, 11% of our *Sall2*-deficient mice showed background-specific neural tube defects. To investigate whether Sall4 may compensate for the absence of Sall2, we generated compound *Sall2* knockout/ *Sall4* genetrap mutant mice. In these mutants, the incidence of neural tube defects was significantly increased. Furthermore, we found a similar phenotype in compound *Sall1/4* mutant mice, and in vitro studies showed that SALL1, SALL2 and SALL4 co-localize in the nucleus. We therefore suggest a fundamental and redundant function of the SALL proteins in murine neurulation.

HIF-1a gene polymorphisms in thyroid cancer. *M. Lu, P. Hsiao, F. Chiang, S. Juo* Grad Inst Medical Genetics, Kaohsiung Medical Univ, Kaohsiung, Taiwan.

Thyroid cancer is the most common endocrine malignancy. Angiogenesis is important for the development of thyroid cancer. HIF-1a(hypoxia-inducible factor)is the headstream of angiogenic pathway. We conducted a case-control study to evaluate the genetic effect of HIF-1a and thyroid cancer. A total of 299 cases and 477 controls were recruited for this study. The genotypes were determined by the Taqman 5 nuclease assay. A total of six tagging SNPs and two non-synonymous SNPs were tested. Hardy-Weinberg equilibrium was tested for each SNP, and the genetic effects were evaluated by X2 test. Individual haplotype pair was predicted by the PHASE 2.0 software. Haplotype-specific and global p-value were obtained by the Hap-Clusting program. None of the single SNP was found to be related with thyroid cancer. Using the variable sliding windows for haplotype analysis, we found the most significant result of a global p value of 4.46 x 10-10 from the combination of SNPs rs966824, rs2301112 and rs2301113. The haplotype CAC yielded the most significant result among these five haplotypes (p-value = 2.21 x 10-5). This haplotype is located within the ODD (oxygen-dependent degradation) domain of HIF-1a gene. The haplotypes comprising from the SNPs rs966824, rs2301112 and rs2301113, which is involving the ODD domain formation, may play a role in the development of thyroid cancer.

Alteration of X-linked gene expression in a novel X-Linked Mental Retardation syndrome. *T.-J. Chen[1], TL. Lee[2], Y. Wang[1], Z. Chen[3], W.-Y. Chan[2], C. M. Tuck-Muller[1], J. E. Martinez[1]* 1) Dept Medical Genetics, Univ South Alabama, Mobile, AL; 2) Section on Developmental Genomics, Lab of Clinical Genomics, NICHD, NIH, Bethesda, MD; 3) Dept. of Human Genetics, UCLA, Los Angeles, CA.

In a previous report, we defined a novel XLMR syndrome in a family with six affected males with mental retardation, muscle atrophy, pigmentary abnormalities and ptosis, and two obligate carrier females in three generations. The defective gene was mapped to the region Xp11-Xp22 by linkage analysis using STR markers. To identify the defective genes for the family, oligo-Array CGH was performed. No deletion or duplication was detected in the probands of either family. To rule out a small deletion and/or duplication, X chromosome tiling array analysis was carried out. Although suspected deletions were detected, these deletions could not be confirmed by PCR analysis. The expression profile of X-linked genes in the probands lymphoblasts was surveyed by X-linked gene cDNA array. The cDNA array contained all known genes on the human X and Y chromosome with controls. The difference in expression level was determined by comparing the ratio of the experimental sample to the normal sample. The expression of at least 50 genes was reduced significantly (>3 fold). The other 21 genes had significantly increased expression (>3 fold). Among these differentially expressed genes, 6 known XLMR genes, 2 XLMR candidate genes and 2 transcriptional factor genes were up regulated, while 12 known XLMR genes, 5 XLMR candidate genes, and 8 transcriptional factor genes were down regulated. It is note worthy that expression of FMR1 and DMD genes decreased more than 10 fold. Thus, we hypothesized that the defective gene for this novel XLMR disorder encodes a transcriptional factor. Interestingly, reduced expression of the FMR1 and FMR2 genes and increased expression of UBE2A, SYP, and SYN1 genes could be associated with mental retardation and CNS anomalies in the XLMR family, while reduction in expression of DMD and increase in expression of the SMAP gene may be the cause of the muscle atrophy and ptosis. Alteration of expression of multiple transcriptional factor genes might result in other clinical abnormalities.

The Adaptor-related Protein complex 2, alpha 2 subunit (AP22) gene is a novel PPAR target gene. *N. Buroker[1], J.-Y. Huang[2], J. Barboza[1], X.-H. Ning[1], M. Portman[1]* 1) Dept Cardiology, Seattle Children's Hosp Res Inst, Seattle, WA; 2) Dept Pediatrics, School of Medicine, UW, Seattle, WA.

The peroxisome proliferator-activated receptors (PPARs) and the retinoid X receptors (RXRs) are members of the nuclear receptor superfamily, which consists of a large number of special transcription factors whose activities are regulated by their cognate ligands. These steroid hormone receptors are important regulators of gene expression and differentiation. These receptors form homo- (RXR) and hetero- (PPAR-RXR) dimers that bind DNA at various response elements (PPAR-RE and RXR-RE). We identify the PPAR/RXR REs in the promoter of the adaptor-related protein complex 2, alpha 2 subunit (AP22) gene. In this study a known PPARactivator (Wy14643) and DMSO (vehicle) was introduced into control and 337T thyroid hormone receptor (TR) transgenic mice. The 337T TR transgenic mouse has been created to reproduce the human genetic disease known as resistance to thyroid hormone (RTH). Heart tissue was extracted and AP22 expression was compared using Affymetrix 430_2 expression arrays and qRT PCR among four studies groups [control, control with Wy14643, 337T TR and 337T TR with Wy14643] consisting of seven mice per group. The gene expression of AP22 in the Wy14643 control and transgenic mouse groups was significantly up-regulated over the vehicle mouse groups for both the array ($p<0.01$) and qRT PCR ($P<0.01$) studies. Duplex oligo DNAs containing the PPAR/RXR motif (aggtca/tccagt) from the AP22 promoter were used in EMSA to verify binding of the PPAR and RXR receptors to their response elements. pGL4.0 [Luc] constructs of the AP22 promoter with and without the PPAR/RXR motifs were co-transfected with mouse PPAR , , or 1 into HepG2 cells and used in lucerifase assays to verify gene activation. In conclusion our study revealed that PPARregulates the mouse cardiac AP22 gene in both the control and transgenic mouse.

Discovery of rare variants via sequencing: Implications for association studies. *S. M. Leal, B. Li* Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Common diseases can be due to functional variants with a wide spectrum of allele frequencies. To understand the architecture of allele frequencies and more importantly provide a biomedical resource for common diseases, the 1000 genome project is initially sequencing ~1,000 individuals from 10 different ethnic backgrounds. Currently genome-wide association studies use tagSNPs to uncover genes for common diseases caused by common variants. In the future there will be a shift to analyzing sequence data to understand the etiology of common diseases due to rare variants. We determined for a set sample size what proportion of rare variants can be identified. For a gene with 20 rare variants each with a frequency 0.1% if 1,000 individuals are sequenced the probability is 0.055, 0.956 and 0.999 that 100%, 75% and 50% of the variants will be uncovered. If only 200 individuals are sequenced these probabilities decrease to $2.3x10^{-10}$, $1.5x10^{-4}$ and $8.6x10^{-2}$, respectively. If each of the 20 variants are functional and have a genotypic relative risk of 5.0; sequencing 200 cases will identify 100% and 50% of the variants with probability $4.6x10^{-9}$ and 0.197, respectively. Sequencing genomes of 1,000 individuals from the same population will reveal ~87% of the variants with frequency of 0.1% with a power of 0.9; the number of identified variants falls to ~18% if only the genomes of 100 individuals are sequenced. The proportion of identified variants will be lower if variant frequencies are <0.1%. For the 1,000 genome project due to ethnic specific sample sizes many rare variants including those involved in disease etiology may not be observed. It is also not advisable to sequence a sample subset in order to discover rare variants and then genotype the remaining sample, since missing variants will dramatically reduce power of association studies.

Gene regulation in primates evolves under tissue-specific selection pressures. *R. Blekhman[1], A. Oshlack[2], A. E. Chabot[1], G. K. Smyth[2], Y. Gilad[1]* 1) Human Genetics, University of Chicago, Chicago, IL; 2) Walter and Eliza Hall Institute of Medical Research, Parkville, Australia.

A long standing hypothesis is that changes in gene regulation play an important role in adaptive evolution, notably in primates. Consistent with this theory, the past decade of research has yielded an increasing number of cases where regulatory changes contribute to species-specific adaptations and to reproductive isolation. To identify adaptive regulatory changes in humans, we performed a genome-wide survey for genes whose regulation evolves under natural selection. To do so, we used a multi-species microarray to measure gene expression levels in livers, kidneys, and hearts from six humans, chimpanzees, and rhesus macaques. Using this comparative expression data, we identified a large number of genes, as well as specific pathways, whose regulation has likely evolved under stabilizing or directional selection. Among the latter set, we found an enrichment of genes involved in metabolic pathways, consistent with the hypothesis that shifts in diet underlie many regulatory adaptations in humans. Finally, we found clear evidence for tissue-specific selection pressures, as well as lower rates of protein evolution for genes whose regulation evolves under natural selection. These observations provide strong support to the notion that adaptive circumscribed changes in gene regulation have fewer deleterious pleiotropic effects compared with changes at the protein sequence level.

Comprehensive Polymorphism Analysis by Haplotype-Specific Extraction. *C. Hellbusch[1], H. Polin[2], M. Danzer[2], C. Gabriel[2], K. Lennartz[1]* 1) QIAGEN GmbH, Hilden, Germany; 2) Österreichisches Rotes Kreuz, Linz, Austria.

For molecular typing of Human Leukocyte Antigens (HLA) and many other genotyping applications the determination of allelic polymorphism combinations is important. Several methods including sequence-specific priming (SSP) or sequencing-based typing (SBT) can be employed but may result in ambiguous typing results, due to two or more different polymorphic allele combinations or new alleles. These can be resolved by haplotype-specific extraction (HSE). This unique method physically separates diploid genomic DNA into its haploid components. Data presented here show the comprehensive analysis of HLA loci by HSE performed with the QIAGEN EZ1 HaploPrep Kit*.

The extraction of the selected alleles is performed fully automated on a QIAGEN EZ1 workstation. After a probe-dependent hybridization and labeling process the targeted allele is subsequently coupled to magnetic beads for separation from nontargeted DNA. The HaploPrep probes show single-base specificity and provide high capture efficiency of the target DNA. Following HSE, analysis of the haplotypes of the targeted genes by SBT leads to clear sequences and thereby to an unambiguous allele identification.

Therefore, HSE represents a fast and effective method for improving genetic analysis in complex genetic contexts. It has been shown to work in combination with various techniques including conventional PCR and sequencing approaches, Pyrosequencing, real-time PCR, and restriction fragment length polymorphism (RFLP) analysis. With the rapidly increasing knowledge about polymorphism associated conditions and diseases, HSE may be used in the future for additional applications, e.g. pharmacogenetics or oncology testing.

* For Research Use Only. Not for use in diagnostics procedures. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

Sex-specific genetic effect of phosphodietserase 4D (PDE4D) on carotid atherosclerosis. *Y. Liao[1, 2], H. Lin[3, 5, 7], M. Yu[6], C. Liu[3,7], S. Juo[1, 3, 4]* 1) Graduate Institute of Medical Genetics, Kaohsiung Medical University, Taiwan; 2) Graduate Institute of Medicine, Kaohsiung Medical University, Taiwan; 3) Department of Neurology, Kaohsiung Medical University, Taiwan; 4) Center of Excellence for Environmental Medicine, Kaohsiung Medical University, Taiwan; 5) Department of Neurology, Hsiao-Kang Hospital, Kaohsiung, Taiwan; 6) Hepatobiliary Division and Department of Preventive Medicine, Kaohsiung Medical University Hospital, Taiwan; 7) Department of Neurology, Kaohsiung Medical University Hospital, Taiwan.

Background and Purpose The phosphodiesterase 4D(PDE4D) gene was reported as a susceptibility gene to stroke. The genetic effect might be attributed to its role in modulating the atherosclerotic changes in carotid artery. Using carotid intima-media thickness(IMT) and plaque index as phenotypes,this study sought to determine the influence of this gene on subclinical atherosclerosis. **Methods** Carotid ultrasonography was performed in 1013 stroke-free subjects age=52.612.2;47.6% men). Genotype distributions were compared among high-(plaque index4),low-risk(index =1-3),and reference(index =0) groups. We analyzed continuous IMT data and further dichotomized IMT using mean plus one standard deviation as the cutoff level. Rs702553 at the PDE4D gene was selected because it confers a risk for young stroke in our previous report. Previous young stroke data with additional 532 control subjects without ultrasonic data were shown as a cross-validation for genetic effect. **Results** The TT genotype was more common in men with a plaque index greater than four(20.0% in TT vs. 9.7% in AA+AT,p=0.008). Using dichotomized IMT data, men with the TT genotype had an odds ratio(OR) of 2.10 (p=0.032) for increased IMT at common carotid artery compared with AA+AT genotypes. In women, neither IMT nor plaque index was associated with rs702553. Sex-differential effect was also found in young stroke (OR=1.92,p=0.02 for men and 1.44,p=0.33 for women). **Conclusions** The present study demonstrates a sex-specific effect of PDE4D on IMT,plaque index and stroke, which highlights its influence on various aspects of atherogenesis.

The *APC* variant E1317Q predisposes to colorectal adenomas by relaxing the target for tumourigenic somatic APC mutations. *D. Azzopardi[1], A. R. Dallosso[1], S. Jones[1], V. Moskvina[2], N. Al-Tassan[1], G. T. Williams[3], S. Idziaszczyk[1], D. R. Davies[4], P. Milewski[5], S. Williams[5], J. Beynon[6], J. R. Sampson[1], J. P. Cheadle[1]* 1) Medical Genetics, Cardiff University, United Kingdom; 2) Biostatistics & Bioinformatics Unit, Cardiff University, United kingdom; 3) Department of Pathology, Cardiff University, United Kingdom; 4) Caerphilly District Miners Hospital, St Martins Road, Caerphilly, United Kingdom; 5) Pembrokeshire and Derwen NHS Trust, Withybush Hospital, Fishguard Rd, Haverfordwest, United Kingdom; 6) 6Department of Colorectal Surgery, Singleton Hospital, Sketty Lane, Swansea, United Kingdom.

Multiple rare non-synonymous variants in *APC* may play a role in inherited predisposition to colorectal adenomas. The mechanisms through which these variants might act remain unclear. It has been proposed that a specific level of -catenin signalling is required for colorectal tumour formation possibly mediated by selection for genotypes retaining one, or rarely two, 20 amino acid -catenin down regulating repeats (20AARs). We investigated the mechanism through which the non-synonymous variant E1317Q, that is located between the first and second 20AARs in *APC*, contributes to tumourigenesis. Comparison of the somatic mutation patterns in *APC* found in tumours from attenuated familial adenomatous polyposis patients that did (Family B) or did not (Patient S) co-inherit E1317Q highlighted significant differences between these tumours, with only 8.2% of tumours carrying E1317Q having somatic mutations predicted to result in mutant polypeptides retaining a single 20AAR, as compared to 61.5% of those which did not carry this variant ($P=9.16x10^{-9}$). *In vitro* assays and showed that E1317Q significantly impaired -catenin regulated transcription when expressed with weak truncating mutations within the -catenin down regulating domain ($P<0.05$). These data suggest that E1317Q relaxes the target for tumourigenic somatic *APC* mutations through its own effects on -catenin-associated signalling.

The genetic association of STAT4 with SLE occurs through independent effects. *A. M. Delgado-Vega[1], A. K. Abelson[1], S. Kozyrev[1], E. Sanchez[2], R. Velasquez[3], N. Eriksson[1], J. Wojcik[4], L. M. V. Prasad[1], G. Lima[5], S. D'Alfonso[6], S. Migliaresi[7], V. Baca[8], T. Witte[9], N. Ortego-Centeno[10], H. Abderrahim[4], B. Pons-Enstel[11], C. Gutierrez[12], A. Suarez[12], J. Martin[2], M. E. Alarcon-Riquelme[1]* 1) Uppsala U, Uppsala, Sweden; 2) Inst Parasitologia y Biomedicina Lopez-Neyra, Granada, Spain; 3) Inst Nal Medicina Genomica, Mexico city, Mexico; 4) Merck Serono, Geneva, Switzerland; 5) Inst Nal C.Medicas y Nutricion Salvador Zubiran, Mexico city, Mexico; 6) U of Eastern Piedmont, Novara, Italy; 7) Second U of Naples, Naples, Italy; 8) CMN Siglo XXI, Mexico city, Mexico; 9) Medical School Hannover, Hannover, Germany; 10) Hospital Clínico San Cecilio, Granada, Spain; 11) Sanatorio Parque, Rosario, Argentina; 12) U de Oviedo, Oviedo, Spain.

Aim. To confirm the genetic association of STAT4 with SLE in 5 independent sets of cases and controls. Methods: We typed 30 tag SNPs in 390 Spanish cases and 480 controls covering the STAT1-STAT4 region. SNPs surviving correction for multiple tests were typed and tested for association in 1581 cases and 1844 controls using real-time PCR. The predictive ability of STAT4 plus IRF5 SNPs was measured by the area under the ROC curve (C-Statistic). Expression and alternative splicing analysis was performed on PBMC. Results. After fine mapping, 4 SNPs remained significant and 3 had independent effects from rs7574865, having rs3821236 the strongest one. Association was replicated in separate sets of cases and controls, except for German and Mexican pediatric sets. High levels of STAT4 expression correlated with rs3821236, rs3024866 and rs7574865, all associated with SLE. The C-statistic increased when rs7574865 and rs2070197, the IRF5 risk haplotype tag, were included in the model compared to rs2070197 alone. No interaction was observed suggesting an additive effect. Conclusions. These data confirm STAT4 as a susceptibility gene for SLE with several independent gene effects, show a strong association signal located around intron 16 62kb from intron 3 and rs7574865, and suggest the presence of functional variants affecting levels of STAT4. Our results also suggest that STAT4 and IRF5 act additively to increase risk for SLE.

Analysis of human urea and nitric oxide cycle gene expression in a panel of normal human tissues. *M. Neill*[1], *M. Summar*[1, 2] 1) Dept Human Genetics, Vanderbilt Univ, Nashville, TN; 2) Dept Pediatrics, Vanderbilt Univ, Nashville, TN.

The urea cycle enzymes serve three important roles. First they are responsible for the processing of nitrogen to urea. The urea cycle is the only metabolic pathway capable of disposing of waste nitrogen. These enzymes also play a role in the amount of nitric oxide produced by the endogenous citrulline-NO cycle. Finally these enzymes are involved in arginine and citrulline production. Given the three distinct roles of these enzymes we postulated that these enzymes might be expressed in a tissue-specific manner. In this study, we have examined the expression of genes encoding the urea cycle and nitric oxide cycle enzymes in a panel of normal human tissues. mRNAs encoding carbamoyl phosphate synthetase (CPSI), n-acetyl glutamate synthase (NAGs), ornithine transcarbamoylase (OTC), argininosuccinate synthetase (AS), argininosuccinate lyase (AL), arginase I (ARG1), arginase II (ARGII), ornithine amino-transferase (OAT), citrin, ORNT1, endothelial nitric oxide (eNOS), inducible nitric oxide (iNOS) and neuronal nitric oxide (nNOS) were amplified by quantitative real-time reverse transcription-PCR assays (qRT-PCR)and normalized to a reference mRNA (GAPDH). We provide evidence that RNA from small intestine, ileum, liver, pancreas, kidney, brainstem, testis, skeletal muscle, spleen, and lung tissue can be amplified by qRT-PCR. We show that patterns of tissue expression for these genes are pedictive of the role of the tissue in either: urea production, citrulline/arginine export, nitric oxide production, or combinations of the above. These findings indicate that the genes involved in the urea and nitric oxide cycles are subject to tissue specific gene regulation. Given these findings it is reasonable to look for shared promoter cassettes amongs these genes which govern their tissue specific expression.

Two cases of mantle cell lymphoma with normal chromosomes 11 and 14 by G-banding but IgH/CCND1 gene fusion by FISH. *J. Xu[1], I. Chin-Yee[2], J. Mangel[2], K. Howson-Jan[2], C. Hamm[3]* 1) Cytogenetics, London Health Sciences Centre and University of Western Ontario, Canada; 2) Hematology, London Health Sciences Centre and University of Western Ontario, Canada; 3) Windsor Regional Cancer Centre, Canada.

Case 1. A 46-year-old male had monoclonal B cells representing ~60% of total leukocyte population in the bone marrow and being CD19/CD5, CD23, FMC7, and CD20 positive and expressing Kappa light chains. The findings suggested possibility of small cell lymphocytic lymphoma, chronic lymphocytic leukemia or mantle cell lymphoma (MCL). Routine G-banding showed a normal male karyotype, 46,XY[50]. However, FISH showed 72% of 200 interphase nuclei with one IgH/CCND1 fusion signal. No FISH signals of IgH/CCND1 fusion were detected in the metaphase chromosomes 11 and 14 examined. In light of the FISH result, this case was interpreted with a final diagnosis of MCL. Case 2: A 62-year-old male was found to have a small monoclonal population of cells (7%) in the bone marrow expressing CD19/CD5, CD20, FMC7 and Kappa light chains. This finding suggested a B-cell lymphoma, possibly MCL. G-banding analysis showed 45,X,-Y[3]/46,XY[23]. The loss of the Y is likely an age-related random event. FISH showed two IgH/CCND1 fusion signals in 14% of 200 interphase nuclei, which confirmed a clinical diagnosis of MCL. This was in keeping with the histology and immunophenotyping seen on his inguinal lymph node biopsy. Notably both of our cases showed that the cells bearing diagnostic IgH/CCND1 fusion were non-dividing in cultures and therefore were missed by routine G-banding and only detected by FISH. At least 22 cases of MCL were reported to have atypical cytogenetic presentations, including 1) absence of chromosomal t(11;14)(q13;q32) in metaphase cells but presence of IgH/CCND1 fusion in interphase cells; and 2) presence of the gene fusion as a result of micro-insertion of CCND1 into the 14q32/IgH locus in apparently normal chromosome 14. Our data adding to the literature emphasize that FISH or other molecular methods must be considered for accurate diagnosis of MCL cases with a normal karyotype by G-banding.

Centromeric DNA break in two reciprocal translocation carriers detected during pre-implantation genetic diagnosis (PGD). *J. Wang, J. Szymanska, R. Habibian, L. Dong, D. Fisher, S. Kou, Q. Huang, J. Chang, A. Hajianpour*
Genzyme Genetics, Monrovia, CA.

Balanced reciprocal translocation carriers are at risk for SAb or abnormal liveborn. Application of PGD by FISH can decrease the risk of these adverse outcomes by transferring only embryos having balanced chromosome (chr) constitution. We have adopted a pre-PGD FISH work-up protocol to select the appropriate probes for PGD that will detect all abnormal segregants. During these processes, we found two cases with unexpected breaks in the centromeric satellite DNA. Case 1 was originally reported as 46,XX,t(17;19)(p11.2;p12). Initial probes selected for pre-PGD work-up were tricolor 17pter, 19pter, and 17cen. The 17cen probe signal was found on both der(17) and der(19), indicating a break in the chr 17 satellite DNA, making this probe set unsuitable for PGD. Subsequently, the chr 19 centromere was also found on both der(19) and der(17). Apparently, both centromeres were split in this true whole arm translocation. The karyotype was revised to 46,XX,t(17;19)(p10;q10). Case 2 was originally reported as 46,XX,t(4;18)(q13;q11.2). Initial probes selected were 4qter, 18qter, and 18cen. The 18cen signal was found on both der(18) and der(4), indicating a break in chr 18 satellite DNA. The der(4) contains a portion of the chr 18 satellite DNA at 4q13, thus represents a dicentric chr. The karyotype was revised to 46,XX,t(4;18)(q13;q10). To date, only four cases of centromere breakage involving chrs 1, 16, and 18 have been reported in reciprocal translocations. Our serendipitous finding of such breaks in two cases, together with the proposition that breakage within the centromere (centromere misdivision, the McClintock mechanism) may be the most common mechanism for ring chr formation, suggests that centromere break may not be rare. These cases provide evidence that break or loss of centromeric satellite DNA does not impair centromere function. A systematic search for centromeric break in whole arm translocations and in reciprocal translocations in which one or both breakpoints are close to the centromere may provide further insight to centromere function and interaction.

Alternative Splicing of the Polycystic Ovary Syndrome (PCOS) susceptibility locus D19S884 in a mini-gene system. *C. Ackerman, A. Biyasheva, M. Urbanek* Department of Medicine. Feinberg School of Medicine, Northwestern University, Chicago, IL.

PCOS is a complex genetic disease that is one the most common causes of infertility and is defined by hyperandrogenemia and irregular menses. In addition to reproductive issues, PCOS is also associated with obesity, insulin resistance and increased risk of developing cardiovascular disease and type 2 diabetes. We have identified a PCOS susceptibility allele within a dinucleotide repeat $(CA)_n$, D19S884, in intron 55 of the fibrillin-3 (*FBN3*) gene on chromosome 19p13.2 that is associated with reproductive and metabolic phenotypes in affected women. Other dinucleotide repeat polymorphisms mapping to noncoding regions have been shown to act as intronic splicing enhancers or silencers, depending on their location and proximity to splicing recognition sequences. Therefore D19S884 may be important in alternative splicing or regulation of *FBN3* transcript levels. We generated mini-gene constructs of the *FBN3* genomic fragment containing D19S884 variants and flanking sequences. We PCR amplified the region containing D19S884 and inserted the products into a splicing reporter vector. A construct was generated for each of the 14 variants at D19S884 that has been observed in women with PCOS, including the PCOS susceptibility allele A8 $(CA)_{17}$. Constructs were transiently transfected into COS-7 cells, RNA isolated, and splicing efficiency assessed by RT-PCR. All variants except A8 demonstrated 100% splicing efficiency. While A8 showed some of the expected splice product, a large percentage of the transcript was not spliced. Surprisingly, A7 and A9, which differ from A8 by one CA repeat, showed normal splicing. The only difference between the A8 mini-gene construct and the other variants is the number of CA repeats. This indicates that D19S884 A8 transcripts have a defect in splicing that decreases the amount of normally spliced transcript and may reduce protein expression. This reduction in fibrillin-3 expression may confer susceptibility to PCOS. The mechanism of the splicing defect and whether this finding reflects the splicing pattern in the complete *FBN3* gene remains to be elucidated.

Prenatal testing of a novel EXT2 nonsense mutation in a Chinese family with hereditary multiple exostoses. *S.-J. Song[1], N. Zhong[1,2]* 1) Peking University Center of Medical Genetics, Beijing, China; 2) Department of Human Genetics, New York State Institute for Basic Research in Developmental Disabilities, Staten Island, NY.

Objective: Hereditary multiple exostoses (HME) is a genetically heterogeneous autosomal dominant disorder, characterized by multiple bony outgrowths. The objective of this study was to investigate the genetic basis of a Chinese family with HME in order to perform prenatal testing for HME in a fetus at 50% risk. Method: Haplotype analyses were performed with polymorphic microsatellite markers at three reported loci (EXT1, EXT2, and EXT3) associated with HME. Screening of the EXT1 and EXT2 genes was performed by direct sequencing of PCR fragments that covered the entire cDNA, following which prenatal testing of the fetus of the proband was performed. Results: It was determined that the EXT3 locus did not link to the family. DNA analysis of EXT1 and EXT2 revealed a novel nonsense mutation (c.1006C>T) in EXT2, which converts the Gln codon (CAA) to the termination codon (TAA) at codon 336 (Gln336X). Prenatal testing showed that the fetus was normal. Conclusion: A novel nonsense mutation in EXT2 was identified in a Chinese family with HME. This result extends the mutation spectrum of EXT2 and can be used for prenatal testing in HME families.

Proteomic Study of Hutchinson-Gilford Progeria Syndrome (HGPS). *L. Wang[1], N. Zhong[1,2]* 1) Peking University Center of Medical Genetics, Beijing, China; 2) Dept. Human Genetics, New York State Institute for Basic Research in Developmental Disabilities.

Hutchinson-Gilford progeria syndrome (HGPS) is a rare genetic disorder that is characterized by segmental premature aging. It is caused by mutations in LMNA, the gene encoded an A-type nuclear lamin protein, lamin A/C. The most frequently occurred mutation is a single nucleotide change in exon 11 that results in a cryptic splice site and the subsequent loss of 50 amino acids within the carboxyl terminus of the lamin A/C proteins. The newly generated mutant lamin A, termed as progerin, may interfere with the nucleic structure and result in bleb formation of nuclei. Presently, the exact pathogenic mechanism of HGPS has not been elucidated. Earlier we have proposed the mutant progerin may have a dominant negative effect on the normal allele of lamin A/C. In this study, we have undertaken a further investigation to study if the expression of progerin may generate alteration of differential expression of proteins in HGPS. Proteomic approach with PF2D system was applied to identify differentially expressed proteins in HGPS cells. Twenty-six proteins down-regulated and four proteins up-regulated were identified. These proteins were classified into five groups: DNA transcription and translation, cell cytoskeleton, cell signal transduction, energy metabolism and cell cycle. Among these proteins, five proteins belonged to calcium-binding protein. Six proteins we interested were selected and validated in HGPS cells. Our study has opened a new avenue for studying the pathogenic mechanism underlying HGPS.

Mutation Spectrum of Kallmann Syndrome. *S. Sha[1], N. Zhong[1,2]* 1) Peking University Center of Medical Genetics, Beijing, China; 2) New York State Institute for Basic Research in Developmental Disabilities, Staten Island, NY.

Kallmann syndrome (KS) is a clinically and genetically heterogeneous disorder, which is characterized by the association of idiopathic hypogonadotropic hypogonadism (IHH) and anosmia or hyposmia. Mutations underlying KS have been identified in four genes KAL1, KAL2, KAL3 and KAL4 that account for nearly 30% of all KS cases. In this study, we have investigated the mutation spectrum among 35 Chinese KS patients with a molecular approach of combining PCR-sequencing and multiplex ligation-dependent probe amplification (MLPA). PCR-sequencing was employed for mutation screening among coding regions of KAL1, KAL2, KAL3 and KAL4 genes. Three point mutations, including two novel mutations K329N and A466V, and one known mutation E514K, were identified in KAL1 gene, which are localized in exon 7, 10 and 11, respectively. These mutations are located in FnIII-2 domain and in the linker region between FnIII-3 and FnIII-4 domains of KAL1 gene, and may be involved in influencing axon branching. MLPA was used to screen for microdeletion or microduplication of KAL1, KAL2, OA1, STS, NELF, GNRHR, GPR54 and GNRH1 genes. Microdeletions, including eight in KAL1 gene and three in KAL2 gene, were identified. In addition, duplications were also found in KAL1 and KAL2 genes. Our results suggest microdeletions and/or microduplications are the predominant genetic defects underlying KS. We recommend that a quantitative analysis such as MLPA should be applied to analyze KS in addition to directly sequencing KAL genes.

MUC20, a novel partner of anosmin-1, possibly involves in olfactory axon branching in Kallmann syndrome. *N. Zhong[1,2], S. Sha[2]* 1) Dept Human Genetics, New York State Inst Basic Res, Staten Island, NY; 2) Peking University Center of Medical Genetics, Beijing, China.

Genetic defect of anosmin-1 has been determined to associate with heterogeneous X-linked Kallmann syndrome (X-KS). However, the detailed pathogenic mechanism is yet unclear. In this study, we have employed the anosmin-1, which is encoded by the KAL1 gene, as the bait in a yeast two hybrid (Y2H) system to screen a human embryonic cDNA library to identify interactive partner(s) for anosmin-1. Our results showed that 16 candidates were identified. Among which, LASS2, L14, MUC20 and FGFR1 were verified by pulling-down experiments and interaction between MUC20 and anosmin-1 was further confirmed by co-immunoprecipitation and double-staining. Both MUC20 and anosmin-1 were detected in human embryonic brain tissues of 18-week, in addition to that anosmin-1 may be detected in 32-week human fetal brain and MUC20 in rat E18 brain by western blots. Truncation analysis showed that MUC20 associates with FnIII-3 and/or FnIII-4 domains but not FnIII-0, FnIII-1 or FnIII-2 of anosmin-1. Olfactory co-culture with cells that have been transfected with a plasmid expressing exogenous MUC20 showed that MUC20 may induce olfactory axon to branch. This suggested that MUC20 might play a role as a modulator for anosmin-1 in guiding axon branching and migration.

Identification of Genomic Microdeletions and Microduplications Among First-Trimester Miscarriages: Analysis by Cytogenetic Karyotyping, Microsatellite Genotyping and ArrayCGH. *Y.-X. Zhang[1], W. J.[2], B.-L. Wu[3], E. C. Jenkins[2], W. T. Brown[2], N. Zhong[1,2]* 1) Peking University Center of Medical Genetics, Beijing, China; 2) New York State Institute for Basic Research in Developmental Disabilities, Staten Island, NY; 3) Children's Hospital, Harvard University, Boston, MA.

Miscarriage is the spontaneous loss of an embryo or fetus before the 20th week of pregnancy. Mostmiscarriage occurs before the end of first trimester (<13 weeks). Although many risk factors relate to this occurrence, genetic factors play the most important role. Usually chromosomal anomalies, including both numerical and structural abnormalities, underlie the majority of miscarriage etiologies. In this study, we employed a comprehensive approach combining cytogenetic karyotyping, PCR-based genotyping, and microarray-based comparative genomic hybridization (arrayCGH) to analyze chromosomal profiles of 115 first trimester miscarriages of Chinese patients. Seventy cases (61%) were found to have chromosomal anomalies, including 90% numerical and 10% structural. Cytogenetic karyotyping, PCR assay, and arrayCGH identified 78.6% (55/70), 2.9% (2 triploids), and 18.6% (13/70) of the anomalies, respectively. The advantage of using this combination approach is that microsatellite genotyping and arrayCGH can be accomplished in spite of culture failure and maternal cell contamination. In addition, arrayCGH can detect the submicroscopic chromosomal anomalies and gene dosage alterations. In this study, we demonstrated a microdeletion of 108 Kb and microduplications of 300-1,460 Kb.

Repetitive elements flank both breakpoints of a chromosome 3 inversion in a 3-generation family with short stature. *I. Hansmann, U. Dutta, D. Wand, D. Schlote* Inst Human Gen, Med Biol, Martin Luther Univ, Halle/Salle, Germany.

Chromosomal rearrangements are often associated with a specific phenotype and they are a significant cause of human disorders. Cytogenetic mapping is a powerful tool for identification of such disease genes. Here we report a case of short stature in a girl with a karyotype of 46, XX,inv(3)(p24.1q26.1). Cytogenetic analysis had revealed a familial pericentric inversion 3, being heterozygous in the proband, her mother and grand mother. In order to characterize the breakpoint physically; FISH (Fluorescence- in situ-hybridization) analysis with large YAC (Yeast Artificial Chromosome) and BAC (Bacterial Artificial Chromosome) clones were performed. Four p specific YACs and six BAC clones were used as probes for FISH. YAC clone CEPHy904H0787 (1090 kb) gave a split signal on the metaphase chromosomes of the proband. The split signal indicates that the target sequence carries the inversion breakpoint. Further, two BAC clones RP11666G20 and CTD2007B5 were identified spanning the breakpoint region, assigning the breakpoint to 3p24.1 and thus narrowed down the breakpoint region to 97.5 kb. Out of the 15 YACs and 10 BACs selected on the q arm, YAC CEPHy904G07889 (1610 kb) and BAC clone RP11-12N13 showed a split signal assigning the breakpoint to chromosomal band 3q26.1. Using sub cloned fragments of these BACs as well as Long range PCR products as probes the breakpoints were now located within a region of 3 kb and 5.3 kb on p and q respectively. Analysis of the genomic sequence surrounding the inversion breakpoints revealed 30% repetitive nature of the DNA containing LTR33A, MER67C, L2 and MER67D elements on p region and LTR16C, MER20, MLT2B1, LTR1B, simple repeats and low copy repeats on q region. We determine that the breakpoints occurred between these repetitive regions. The presence of these repetitive elements, especially MER and LTR elements at the junction of the breakpoints suggest that the inversion may be the result of these repetitive elements.

Defining phenotypes in quantitative traits: lessons from the study of myopia. *C. L. Simpson[1], P. Hysi[1], C. J. Hammond[2], P. M. Cumberland[1], J. S. Rahi[1]* 1) Ctr Pediatric Epid & Biostat, Inst Child Hlth, London, United Kingdom; 2) Twin Research and Genetic Epidemiology Unit, Kings College London, London.

Recent technological, computational and statistical advances have made study of complex quantitative traits more feasible. However, less attention has been paid to the importance of misclassification of phenotype which might lead to systematic bias and thus erroneous findings in genetic studies. Refractive error is the most common cause of reduced vision worldwide. It is an archetypal complex quantitative trait, although its highly skewed and leptokurtotic distribution poses additional analytical challenges. In epidemiological studies, this trait is frequently analysed as a categorical trait, most commonly by dichotomising into myopia versus normal refractive status, but without consensus on thresholds for classification. The purpose of this study was to investigate whether use of different clinically relevant myopia categories changed the results of linkage and association studies compared to quantitative trait analysis. We used both simulated datasets and real data from on-going genetic investigations of refractive error, including data from the UK Twin Study for linkage and from the 1958 UK Birth Cohort for association analysis. Varying the phenotype definition changed the numbers of affected and unaffected, as expected. In both the simulated and real datasets, the physical position with the highest LOD score or smallest p value varied significantly with the definition of the phenotype. LOD scores of 1 in the quantitative trait were frequently inflated to much more significant scores of 3 or more. Equally LOD scores of 3 or more in the quantitative trait were lower in the dichotomised traits. Using refractive error as an exemplar, we have shown that observed differences in the findings of linkage or association studies of quantitative traits may be attributable to misclassification of the phenotype which can occur when it is converted into a categorical trait using an arbitrary threshold. We suggest that, wherever possible, preference should be given to using the available quantitative trait measurements, especially if the underlying biology supports it.

Who owns those blood spot samples: Revisited. *A. A. A. Saadallah* NLNBS Genetics, King Faisal Specialist Hospital & Research Center, Riyadh, Saudi Arabia.

Ethics pertains uniformly to the practice of public health, the execution of population programs, and the conduct of research. Newborn screening as public health service is recently spreading to nations outside the developed world as in the region of the Middle East and North Africa. The population of the MENA region has large family size, high level of inbreeding with consanguinity 25-70% and has a high occurrence of inherited metabolic diseases. Polymerase chain reaction technology renders biobanked tissue as blood samples a locale of interest for new modes of research generating a chain of bioethical and legal dilemmas in the West; as weighing the interests of the society and future patients against the interests of individuals in protecting their rights and integrity through proper consideration of adequate information. The matter of patient property of his or her sampled or excised tissue is held by certain bioethicists as inbuilt dilemma in biomedical research that might escalate legally to reach all the way to the Supreme Court. The issues that are under debate in the West will have to be examined as per the ethical standards, customary practices, and standpoints of the non-western countries. This author will attempt to compare and contrast special characteristics of societies in MENA region in relation to those in the West. Because of these predicted variations, the author claims that it is just a matter of time before healthcare providers and policy makers in MENA will confront matters involving societal ethics, education, legality, and legislation in relation to the newly introduced newborn screening programs, and the genetic testing, and subsequent research that will get tempted to use the millions of blood spots biobanked from the screening programs.

Genetic susceptibility to prostate cancer due to rare mutations in genes in the DNA repair pathway. *Z. Kote-Jarai[1], S. Jugurnauth[1], S. Mulholland[1], M. Guy[1], S. Edwards[1], D. Leongamornlert[1], N. Sodha[2], L. O'Brien[1], R. Wilkinson[1], A. Hall[1], D. Dearnaley[2], K. Muir[3], A. Artitaya Lophatananon[3], The. UKGPSC Collaborators[2], The. British Assoc Urol Surgeons[2], D. Easton[4], R. Eeles[2]* 1) Translational Cancer Genetics Team, The Institute of Cancer Research, Sutton, Surrey, UK; 2) The Royal Marsden NHS Foundation Trust, Sutton, Surrey, UK; 3) Univeristy of Nottingham Medical School, Queens Medical Centre, Nottingham ,UK; 4) CR-UK Genetic Epidemiology Unit, University of Cambridge, Cambridge, UK.

The causes of prostate cancer (PrCa) are still not well understood, but there is strong evidence that a proportion of cases occurs due to a genetic predisposition and the associated disease manifests at a young age of onset and/or in familial clusters. Linkage studies suggest multiple candidate loci, but so far none have identified high-risk PrCa predisposition genes. The first convincing high risk PrCa predisposition gene, BRCA2, was identified using a candidate gene analysis approach which showed that about 2% of men who are diagnosed with PrCa at <55 years have a germline mutation in their *BRCA2* gene. Since *BRCA2* has a direct role in DNA damage repair we are investigating the possibility that other genes in this pathway might be involved in PrCa predisposition. We have screened 6 such genes for germline mutations in blood DNA samples from men with PrCa. For the initial screen, the youngest affected individual was selected from 96 UK PrCa families with at least 3 affected individuals. The candidate genes were analysed by direct sequencing. When deleterious mutations were found, the contribution of the mutation to PrCa was established by screening additional DNA samples from familial and young onset patients. Rare mutations and genetic variants have been found in some genes in the DNA repair pathway and this pathway seems to be important in some instances of PrCa. The relative risks are moderate (about 2-3 fold) however these results might be be important in risk prediction, targeted screening and treatment of such individuals.

Towards Predicting Individual Risk for Age-related Macular Degeneration. *K. Spencer[1], L. M. Olson[1], P. Gallins[3], W. K. Scott[3], N. Schnetz-Boutaud[1], A. Agarwal[1], E. A. Postel[2], M. A. Pericak-Vance[3], J. L. Haines[1]* 1) Center for Human Genetics Research, Vanderbilt University, Nashville, TN; 2) Center for Human Genetics, Duke University, Durham, NC; 3) Miami Institute for Human Genomics, University of Miami, Miami, FL.

Though genetic testing is available for many Mendelian disorders, few DNA tests reliably predict an individuals risk for complex genetic diseases. Age-related macular degeneration (AMD), the leading cause of blindness in the elderly, is rare among complex diseases in that evidence for multiple strong genetic and environmental factors has been established. Among these risk factors age, smoking, CFH, Y402H, and the ARMS2/HTRA locus explain a significant portion of disease, raising the possibility for development of a successful predictive algorithm. We used a subset of 352 AMD cases and 184 controls ascertained at Vanderbilt and Duke Universities to create both logistic regression (LR) and decision tree (DT) models of AMD. We then applied these models to an independent dataset of 89 cases and 48 controls also ascertained at Vanderbilt or Duke. The LR and DT agreed in predicted diagnosis ~87% of the time; the remaining 13% of the individuals were not classified. Of those classified, 79% were classified correctly (84.1% of cases and 67.7% of controls). Though these results are very encouraging, applicability of this model to the general population will be important. Efforts to test this algorithm in and independent, diverse cohort are ongoing.

Evidence for association between intron 1 of the follistatin gene *(FST)* and PCOS. *A. Biyasheva[1], R. S. Legro[2], A. Dunaif[1], M. Urbanek[1]* 1) Dept of Medicine, Northwestern University Feinberg School of Medicin, Chicago, IL; 2) Dept of Obstetrics and Gynecology, Pennsylvania State University, Hershey, PA.

Polycystic ovary syndrome (PCOS) is a genetically complex disorder and the most common form of annovulatory infertility among reproductive age women. PCOS is characterized by hyperandrogenemia and irregular menses and is associated with insulin resistance, obesity and increased risk of developing Type 2 diabetes mellitus. In our initial candidate gene screen, the gene encoding follistatin, *FST*, showed the strongest evidence for linkage with PCOS (Urbanek et al 1999 PNAS). However, mutation screening of FST followed by testing for association did not show evidence for association between sequence variation in *FST* and PCOS in our families (Urbanek et 2000 JCEM). Sequence analysis and association studies of *FST* coding variants by other investigators also failed to identify a PCOS-associated variant (Liao et al 2000 Hum Mol Reprod; Jones et al 2007 Hum Mol Reprod). However, we now carried out a broader analysis of the entire genomic region of FST including 20 kb upstream and downstream of the gene and all intronic sequences. An association study of 633 women with PCOS and 574 controls showed strong evidence for association of PCOS with variants in intron 1. The strongest evidence of association was with rs3756498 (?2=14.4, p=0.0001). This study is the largest and most complete analysis of genetic variation of FST in PCOS to date and underscores the importance of screening intronic regions in genetic studies of complex disorders. Follistatin binds and biologically inactivates activin, a member of the TGF superfamily, therefore playing a critical role in gonadal hormone secretion and development and function of the ovary. It is of great interest that FBN3, the gene that encodes fibrillin-3 and is the strongest PCOS candidate gene identified to date, may also act to sequester members of the TGF signaling pathway in a manner analgous to follistatin's sequestration of activin. The fact that the two strongest PCOS susceptibility loci identifed are believed to regulate TGF superfamily signaling, supports a critical role for this pathway in the etiology of PCOS.

Is locus heterogeneity or phenotype misclassification more costly for family-based association analysis? *D. Gordon[1], C. Chen[2], G. Yang[1], S. Buyske[1,2], T. Matise[1], S. Finch[3]* 1) Dept Genetics, Rutgers, State Univ of NJ, Piscataway, NJ; 2) Dept Statistics, Rutgers, State Univ of NJ, Piscataway, NJ; 3) Dept Applied Math Statistics, Stony Brook Univ, Stony Brook, NY.

Background: Both locus heterogeneity and phenotype misclassification can reduce the statistical power to detect association with disease genes in family-based studies. We ask the questions: (1) Is there a way to quantify the sample size increase for a fixed power and significance level due to each phenomenon? (2) Which phenomenon increases sample size more?

Methods: We derive the non-centrality parameter for the transmission disequilibrium test in the presence of locus heterogeneity and phenotype misclassification. This derivation provides an analytic solution to power in the presence of each. We test these calculations with simulation.

Results: The maximum difference between our simulation and analytic results is 0.015, suggesting that our analytic results may be used to design studies of TDT sample size requirements in the presence of locus heterogeneity or phenotype misclassification. Sample size increase is substantially greater for phenotype misclassification than for locus heterogeneity. The relative increase becomes more substantial as the disease prevalence becomes smaller.

Conclusions: While locus heterogeneity is an issue in family-based association analysis, correct classification of phenotype is a relatively more important issue for well-powered studies.

Duplication of ARX and IL1RAPL1 gene in a sex reversal patient with mental retardation. *Y. Wang, C. M. Tuck-Muller, J. E. Martinez, X. Qian, T.-J. Chen* Dept Med Gen, Univ South Alabama, Mobile, AL.

Most of reported cases of interstitial duplications of the short arm of the X chromosome in male have mental retardation and other clinical anomalies. Functional disomy for the dosage sensitive genes within duplication region is believed to play a major role in the phenotype. Most of the reported cases have duplication between Xp22.to pterm. In all of previously reported cases, duplication were determined by G-banding, FISH and linkage analysis. No duplication of individual gene has been directly associated with mental retardation. Here, we reported a sex reversal patient with severe mental retardation due to a duplication at Xp22.11p21.3. The patient is a phenotypic female. At birth, she had ambiguous genitalia and multiple congenital anomalies many, including macrocephaly, left amblyopia and left esotropia. She has developmental delay, seizure, and mental retardation. She has severe behavior problems with a very limit language ability. To precisely determine the duplication regions, high resolution oligo CGH array analysis was performed. The duplication was determined to be about 10 Mb in size from 22.92 Mb to 32.28 mb at hg18. 28 genes are located within this duplication, including about most part of DMD, GK, DAX1, and POLA gene. Two XLMR gene, ARX and IL1RAPL1, also included in the duplication. In a recent report, a smaller duplication, 637 kb, was discovered in two sex reversal sisters. The two sisters has gonadal dysgenesis but do not have mental retardation and other abnormalities. DAX1 and GK genes are included in the small duplication. It is obviously demonstrated that DAX1 cause the sex reversal in the patients of previous report and this report. Genes responsible for the mental retardation in our patient should locate outside the small duplication. Therefore, we hypothesized that ARX or IL1RAPL1 is a dosage sensitive gene. Duplication resulted in mental retardation might be due to the overexpress of either of the two genes. Gene express profile from the patient is under investigation.

Candidate gene association study of vulnerability to develop heroin addiction in African Americans: evidence for association at the glutamate receptor *GRIN2A*. *O. Levran[1], D. Londono[2], K. O'Hara[1], J. Rotrosen[3], P. Casadonte[3], S. Linzy[4], M. Randesi[1], J. Ott[2,5], M. Adelson[4,1], MJ. Kreek[1]* 1) The Laboratory of the Biology of Addictive Diseases , Rockefeller University, New York, NY; 2) The Laboratory of Statistical Genetics, The Rockefeller University, New York, NY; 3) VA New York Harbor Healthcare System and NYU School of Medicine New York, NY; 4) Dr. Miriam and Sheldon G. Adelson Clinic for Drug Abuse Treatment and Research, Las Vegas, NV; 5) Beijing Institute of Genomics, Chinese Academy of Sciences, Beijing, China.

Heroin addiction (HA) is a chronic complex disease with a substantial genetic contribution. This study was designed to identify gene variants predisposing to or protecting from HA in African Americans, with emphasis on candidate genes for addiction. We have performed a case-control association analysis by screening 1350 variants in 130 genes. The sample consisted of 202 former severe heroin addicts in methadone treatment and 167 healthy controls with no history of drug abuse. Single-SNP, haplotype and multi-SNP genotype patterns analyses were performed. Seventeen SNPs, showed the strongest evidence for association with HA (nominal $P < 0.008$ for allelic test). These SNPs are from genes encoding several receptors: adrenergic (*ADRA1A*), arginine vasopressin (*AVPR1A*), cholinergic (*CHRM2*), dopamine (*DRD1*), GABA-A (*GABRB3*), glutamate (*GRIN2A*), and serotonin (*HTR3A*), as well as alcohol dehydrogenase (*ADH7*), glutamic acid decarboxylase (*GAD1* and *GAD2*), the nucleoside transporter (*SLC29A1*), and diazepam binding inhibitor (*DBI*). The most significant result for haplotype association analysis was for *GRIN2A* (nominal $P = 0.000055$, corrected for multiple testing $P = 0.058$). This study corroborates few reported associations with alcohol and drug dependence as well as anxiety disorders, and extends the list of variants with protective and susceptibility effects on heroin addiction, which may be African-specific. Further studies will be necessary to confirm these associations and to elucidate the roles of these variants in drug abuse vulnerability.

Polymorphic *Alu* Insertions and the Genetic Structure of Population Groups from Punjab, Northwest India. *A. Bhanwer, A. Kumar, K. Matharoo, S. Sharma* Human Genetics, Guru Nanak Dev University, Punjab, India.

India presents with a pool of genetically and culturally diverse populations. The Punjab region of India has an extreme importance in understanding the peopling of India as it acted as a corridor to the foreign invaders from Eurasia and Central Asia. Keeping in mind the whole scenario, the present study was designed to explore the genetic diversities and affinities among the seven major ethnic groups: Brahmins, Jat Sikhs, Banias, Rajputs, Sainis, Gujjars and Harijans, from the region. A total of 276 individuals belonging to these population groups were analysed for five *Alu* Insertion/Deletion loci (ACE, PV 92, APO, D1, FXIIIB).

The average (over studied 5 loci) observed heterozygosity was 0.39 in Brahmins, 0.37 in Jat Sikhs, 0.38 in Banias, 0.32 in Rajputs, 0.42 in Sainis, 0.42 in Gujjars and 0.34 in Harijans. The average gene diversity values were 0.44, 0.41, 0.39, 0.41, 0.45, 0.38 and 0.41, respectively for Brahmins, Jat Sikhs, Banias, Rajputs, Sainis, Gujjars and Harijans.

It was observed that each *Alu* insertion was highly polymorphic in all the groups except for *Alu* APO. In Gujjars, the deletion allele for the *Alu* APO was found to be absent while the same revealed low level of polymorphism among the other studied endogamous groups. The overall study favours a deep in time inflow of genes to the region and then their expansion. This was followed by further split of population groups into largely isolated (endogamous) population groups. It is suggested that the structuring of the Indian population groups as various endogamous groups might have occurred within the Indian subcontinent without major influence from any external population source.

Examination of Candidate Genes in Age-Related Macular Degeneration. *L. M. Olson[1], K. Spencer[1], P. Gallins[3], W. K. Scott[3], N. Schnetz-Boutaud[1], A. Agarwal[1], E. A. Postel[2], M. A. Pericak-Vance[3], J. L. Haines[1]* 1) Dept Molec Phys and Biophysics, Vanderbilt Univ Medical Ctr, Nashville, TN; 2) Duke Eye Ctr, Duke University, Durham, NC; 3) Institute for Human Genomics, University of Miami, Miami, FL.

Age-related macular degeneration (AMD) is a complex genetic disorder of the central retina characterized by large soft drusen, geographic atrophy, and/or choroidal neovascularization. Multiple pre-disposing genetic risk loci (CFH , ARMS2/HTRA1, C3) and protective loci (CFHR3/CFHR1 deletion, CFB/CC2) have been identified. However, the additional susceptibility loci with smaller marginal or interactive effects exist. We selected as candidate genes the complement C3 receptor subunit 3 (C3RA), pigment epithelium-derived factor (PEDF), and toll-like receptor 3 (TLR3) based on biological plausibility and/or to replicate previously proposed associations. We tested polymorphisms in these genes in two independent Caucasian datasets: a family-based dataset of 144 multiplex and 79 singleton families, and a dataset of 715 cases and 285 controls. We found no evidence of association for PEDF M72T in either dataset (p0.2 for both). A SNP in C3RA was weakly associated in the family-based dataset (p=0.03), but this effect was not replicated in the case-control dataset (allelic ^{2} p=0.25). Given the known association of C3 R102G with AMD, we tested for a C3-C3RA interaction using likelihood ratio testing, and observed weak evidence for interaction (p=0.07). A SNP in TLR3 was modestly associated in the family dataset (PDTgeno=0.01), and a neighboring SNP in linkage disequilibrium was nominally associated in the case-control dataset (p=0.01). However, after adjusting for known AMD susceptibility factors using logistic regression, neither the effects of C3RA nor TLR3 remained significant. None of the results survived severe or moderate correction for the number of SNPs tested within each gene. We did not detect strong evidence of association in any of the 3 candidates suggesting that it is unlikely that variation within these genes makes a large contribution to the etiology of AMD.

CAG repeat length variation on the background of the POLG gene SNPs. *B. Malyarchuk[1], M. Perkova[1], M. Derenko[1], T. Grzybowski[2]* 1) Institute of Biological Problems of the North, Magadan, Russian Federation; 2) The Nicolaus Copernicus University, Ludwik Rydygier Collegium Medicum, Institute of Forensic Medicine, Bydgoszcz, Poland.

The aim of our study was to investigate the pattern of distribution of the CAG repeats in the POLG gene genotypes and haplotypes. For this purposes, we have analyzed two linked POLG SNPs, in addition to the previously obtained data on CAG repeat length variation in populations of Russians (n=50) and Buryats (n=94) (see Table, where W is 10-CAG repeat, M is not10-CAG repeat, SNP1 is rs2238296, SNP2 is rs758130). Linkage disequilibrium analysis showed almost complete linkage of alleles for these loci in both populations.

POLG genotype and haplotype frequencies in Russians and Buryats

CAG	WW	WW	WW	WW	WW	WW	WM	WM	WM	MM	MM	W	W	W	M	M
SNP1	TT	TC	CC	TT	TC	TT	TT	TC	TT	TT	TC	T	T	C	T	C
SNP2	TT	TC	CC	TC	CC	CC	TT	TC	TC	TT	TC	T	C	C	T	C
RUS	0.22	0.34	0.14	0	0.06	0	0.16	0.02	0.02	0.02	0.02	0.47	0.04	0.35	0.13	0.01
BUR	0.29	0.43	0.11	0.02	0.04	0.02	0.05	0.04	0	0	0	0.54	0.05	0.36	0.05	0

Five haplotypes were either directly observed or inferred using the EM and ELB algorithms. Significant predominance of not-10 CAG alleles was found on TT haplotype. Analysis of nucleotide sequences of the POLG gene haplotypes in humans and chimpanzee has shown that two human haplotypes TT and CC could evolve separately for a long time, with a coalescence time of approximately 1.3 millions of years between them. It is important to note that CAG-repeat instability is associated almost exclusively with younger human haplotype TT. This work was supported by the grant from the FEB RAS (06-3-A-06-176).

Effect of vitamin A deficiency on the epigenomics of male germ cells. *C. N. Boucheron, V. Baxendale, O. M. Rennert, W. Y. Chan* NICHD/LCG/SDG, NIH, Bethesda, MD.

Vitamin A and its derivatives (the retinoids) participate in many physiological processes including vision, cellular differentiation and reproduction. The vitamin A actions in cells occur via the binding of its active metabolite, the retinoic acid, on its nuclear receptors (RARs and RXRs), which in turn regulate the expression of numerous genes. Vitamin A deficiency (VAD) is the leading cause of preventable blindness in children and a factor that increases the risk for severe infections. In addition, VAD affects the process of spermatogenesis, which is the subject of our present study. Spermatogenesis is a highly regulated process of differentiation and complex morphologic alterations that, in the postnatal testis, leads to the formation of sperm in the seminiferous epithelium. VAD induces early cessation of spermatogenesis, characterized by degeneration of all meiotic germ cells, leading to seminiferous tubules containing mostly type A spermatogonia (considered as the stem cells of spermatogenesis) and Sertoli cells. In this study, we want to define the molecular basis of the effect of VAD-induced arrest of spermatogenesis. Our experimental protocol (8-12 weeks of VAD) will allow us to perform transcriptome analysis (SAGE, microarray, RT-PCR), and methylation analysis (tiling array), to compare expression and epigenomic profiles between normal and VAD testis. We hope to identify genes responsible for the effect of VAD on spermatogenesis. *This research is supported by the Intramural Research Program of the NIH, NICHD.*.

-459C>T point mutation in 5' non-coding region of human JGB1 gene is linked to Charcot-Marie-Tooth neuropathy but not -713G>A. *M. Li[1], T. Cheng[2], P. Ho[2], Y. Song[1], S. Ho[2,3]* 1) Department of Biochemistry, University of Hong Kong; 2) Division of Neurology, University Department of Medicine, University of Hong Kong; 3) Research Center on Heart, Brain, Hormone and Healthy Aging, University of Hong Kong.

Charcot-Marie-Tooth (CMT) neuropathy is inherited with high genetic and clinical heterogeneity. The X-linked form (CMT-X) is linked to mutations in GJB1 gene. In order to explore more underling genes and mutations responsible for CMT disease, we conducted genome-wide linkage scan in two CMT pedigrees and mutation screening among subjects from both pedigrees as well as a control group. Subjects of the two pedigrees were genotyped by high-throughput technologies of Illumina and Affymetrix. Gene chip data were integrated and pre-processed by our tool, IGG, for genome-wide linkage analysis. Multipoint genome-wide linkage scan was performed by a 64-bit program, Merlin on a SUN Solaris Operating System. Both non-parametric and parametric models were tried in the linkage scan. A bioinformatics tool, Endeavour, was used to predict and prioritize the candidate genes within the region(s) from our linkage scan for follow-up mutation screening. Genomic DNA was extracted from peripheral blood from subjects by Qiagen Extraction kit according to protocol (Valencia, CA), and tested for mutations in the GJB1 gene by Applied Biosystems 3730xl DNA analyzer. The genome-wide linkage scan in two pedigrees revealed a candidate region, 2.5 cM or so at Chromosome Xq13.1. The total maximum LOD score is 5.68. The GJB1 gene, a known CMT-X gene was found in this region. A genomic variant, -459C>T identified in 5-untranslated region of exon 1 (numbering in relation to the translational [ATG] start codon) of GJB1 was co-segregated with CMT-patients in our pedigrees. But another variant -713G>A of this gene was found in both affected and unaffected in our study, which is inconsistent with a published report in a Taiwanese family. Our results support -459C>T but not -713G>A variant as a causative mutation in CMT-X, which can help clarify the causal mutations of CMT-X in the non-protein coding region of GJB1.

Haplotype analysis of *MHC2TA* and *KIAA0350* on chromosome 16p13 and risk for rheumatoid arthritis (RA). *P. G. Bronson[1], P. P. Ramsay[1], M. F. Seldin[2], P. K. Gregersen[3], L. A. Criswell[4], L. F. Barcellos[1]* 1) Univ of California, Berkeley, CA; 2) Univ of California, Davis, CA; 3) Feinstein Institute for Medical Research, NY; 4) Univ of California, San Francisco, CA.

An association between MHC genes, particularly those within the class II HLA region, and RA is well established, and accounts for ~30% of the genetic component for this disease. The identification of additional RA loci is critical to further our understanding of disease pathogenesis. Two candidate RA genes were chosen for this study based on strong hypotheses: (1) the MHC class II transactivator gene (*MHC2TA*), the most important transcription factor regulating genes required for class II MHC-restricted antigen presentation, and (2) the C-type lectin domain family 16, member A gene (*KIAA0350*), a putative immunoreceptor recently identified as a susceptibility locus for type 1 diabetes (T1D). Both loci are located together on 16p13, a chromosomal region previously implicated in RA linkage studies. Our study sample was comprised of 682 anti-CCP positive RA cases and 752 controls (90% N. European ancestry). We investigated 12 haplotype blocks encompassing 5 *MHC2TA* SNPs and 61 *KIAA0350* SNPs. We used the *haplo.stats* R package to compute MLE of haplotype probabilities with the EM algorithm and score statistics to test for association. Analyses were restricted to haplotypes with frequencies 5%. No association between RA and variation within *MHC2TA* was observed. However, the *KIAA0350* rs2903692-rs17673553 haplotype was associated with RA (global P=0.02); specifically, the *AG* haplotype was over-represented in cases (25.9%) vs. controls (21.5%) (OR=1.3, 95% CI=1.1-1.5, *P*=0.006). Our results suggest, for the first time, that genetic variation in *KIAA0350* contributes to risk for RA. Further studies are warranted to replicate this important result, and to investigate the possibility that *KIAA0350* may confer susceptibility to other autoimmune diseases.

Audioprofile-directed screening identifies novel mutations in human autosomal dominant deafness genes at the DFNA2 and DFNA9 loci. *M. S. Hildebrand[1], D. Tack[2], S. J. McMordie[1], A. DeLuca[2], I. Hur[2], C. Nishimura[1], P. Huygen[3], T. L. Casavant[2], R. J. H. Smith[1]* 1) Department of Otolaryngology, University of Iowa, Iowa City, IA; 2) Department of Electrical and Computer Engineering, University of Iowa, Iowa City, Iowa 52242, USA; 3) Department of Otorhinolaryngology, Radboud University Nijmegen Medical Centre, 6500 HB Nijmegen, The Netherlands.

Rationale: Gene identification in small families segregating autosomal dominant sensorineural hearing loss presents a significant challenge. To address this challenge, we have developed a machine learning based software tool, AudioGene v2.0, to prioritize candidate genes for mutation screening based on audioprofiling. Methods: We analyzed audiometric data from a cohort of 77 American families with high frequency autosomal dominant sensorineural hearing loss. Those families predicted to have a DFNA2 or DFNA9 audioprofile by AudioGene v2.0 were screened for mutations in the KCNQ4 or COCH genes respectively. Results: Two novel missense mutations and a stop mutation were detected in KCNQ4 in three American families predicted to have DFNA2-related deafness for a positive predictive value of 6.3%. The false negative rate was 0%. The missense mutations were located in the channel pore region and the stop mutation was in transmembrane domain S5. The latter is the first DFNA2-causing stop mutation reported in KCNQ4. In one DFNA9-predicted family a known pathogenic missense mutation was detected in the LCCL domain for a positive predictive value of 14.3%. In addition to hearing impairment, affected members of the DFNA9 family also exhibited vestibular dysfunction including a rare inner ear abnormality, superior semicircular canal dehiscence. Conclusions: Our data suggest: (1) mutations at the DFNA2 and DFNA9 loci are a common cause of high frequency, autosomal dominant hearing impairment in the American population; and, (2) that AudioGene audioprofile analysis can effectively prioritize genes for mutation detection in small families segregating autosomal dominant sensorineural hearing loss. AudioGene software will be made freely available to clinicians and researchers once it has been fullly validated.

Genome-wide association study identifies a susceptibility variant for narcolepsy. *T. Miyagawa[1], M. Kawashima[1,2], N. Nishida[1], J. Ohashi[1], R. Kimura[1,3], A. Fujimoto[1], M. Shimada[1], S. Morishita[4], T. Shigeta[4], L. Lin[2], SC. Hong[5], J. Faraco[2], YK. Shin[5], JH. Jeong[5], Y. Okazaki[6], S. Tsuji[7,8], M. Honda[9,10], Y. Honda[10], E. Mignot[2,11], K. Tokunaga[1]* 1) Dept of Human Genetics, Grad Sch of Med, Univ of Tokyo; 2) Center for Narcolepsy, Stanford Univ Sch of Med; 3) Dept of Forensic Med, Tokai Univ Sch of Med; 4) Dept of Computational Biology, Grad Sch of Frontier Sciences, Univ of Tokyo; 5) Dept of Neuropsychiatry, St. Vincents Hosp, The Catholic Univ of Korea; 6) Tokyo Metropolitan Matsuzawa Hosp; 7) Dept of Neurology, Grad Sch of Med, Univ of Tokyo; 8) Center for Integrated Brain Medical Science, Grad Sch of Med, Univ of Tokyo; 9) Dept of Sleep Disorders Research, Tokyo Inst of Psychiatry; 10) Japan Somnology Center, Neuropsychiatric Research Inst; 11) Howard Hughes Medical Inst, Stanford Univ Sch of Med.

Narcolepsy, a sleep disorder characterized by sleepiness, cataplexy and REM sleep abnormalities, is known to be strongly associated with human leukocyte antigen (HLA). However, multiple susceptibility genes in addition to HLA must also participated in the onset, since narcolepsy is obviously a complex disease. We conducted a genome-wide association study using 500K SNPs in 222 Japanese narcoleptics and 389 Japanese controls, with the replication study of top hits in 159 Japanese narcoleptics and 190 Japanese controls, then followed by the testing of 424 Koreans, 785 Caucasians and 184 African Americans. A SNP was significantly associated with narcolepsy in Japanese samples (odds ratio = 1.79, $P = 4.4 \times 10^{-7}$) and Korean samples (odds ratio = 1.40, $P = 0.03$). In Caucasian and African American samples, the same tendency was observed, although the frequency of the risk allele was much lower. The meta-analysis of the four populations gave $P = 5.9 \times 10^{-8}$, with an odds ratio of 1.63. The LD block including this SNP encompassed two genes. Furthermore, real-time quantitative RT-PCR assays showed that the transcription levels of these two genes were significantly lower in individuals possessing the risk allele. The present findings indicate that a polymorphism regulating expression levels of these two genes is a determinant of susceptibility to narcolepsy.

A novel sarcoidosis disease gene with potential relevance for related granulomatous inflammatory phenotypes identified by a genome-wide association study. *S. Hofmann[1], A. Franke[1], A. Fischer[1], G. Jacobs[1], K. Gaede[2], J. Mueller-Quernheim[3], M. Schuermann[4], M. Nothnagel[5], P. Rosenstiel[1], S. Schreiber[1]* 1) Institute of Clinical Molecular Biology, Christian Albrechts University, Kiel, Germany; 2) Leibniz Research Center Borstel, Germany; 3) Medical University Hospital Freiburg, Germany; 4) Institute of Human Genetic, University of Lübeck, Germany; 5) Institute of Medical Informatics and Statistics, Christian Albrechts University, Kiel, Germany.

Sarcoidosis is a complex chronic inflammatory disorder with predominant manifestation in the lung. In the first genome-wide association study (440,000 SNPs) of this disease, comprising 499 German sarcoidosis patients and 490 controls, we detected a series of genetic associations, the most prominent being with the *SARC2** gene. Validation in an independent sample (1,649 cases, 1,832 controls) confirmed the association (SNP rs00*: $P=3.0\times10^{-13}$, rs01*: $P=1.0\times10^{-5}$, allele-based test). Extensive fine mapping located the association signal to a region between exon 5 and exon 14 of *SARC2*. A common non-synonymous SNP (rs02*, TC, p.Arg00*Cys) was found to be strongly associated with sarcoidosis. The GWAS lead SNP and additional risk variants in the region (rs03*, rs04*, rs05*) were in strong linkage disequilibrium with rs02. Sequencing of all exons confirmed rs02 as a putative causative variant and revealed eight novel mutations. The SARC2 protein has complex and essential functions in several biological pathways, including apoptosis and proliferation. We also examined the association of *SARC2* with other granulomatous diseases (e.g. Crohn disease). Data suggest a potential relevance of *SARC2* as a risk gene for phenotypic related disorders and indicate it as a susceptibility locus of general importance. *anonymized, the gene name and SNP identification numbers will be present at the meeting ·

SACGHS policy recommendations for pharmacogenomics and the oversight of genetic testing. *A. Ferreira-Gonzalez, The Secretary's Advisory Committee on Genetics, Health, and Society* Virginia Commonwealth University, Richmond, VA.

The Secretarys Advisory Committee on Genetics, Health, and Society (SACGHS) has completed two reports that provide recommendations for pharmacogenomics (PGx) and the oversight of genetic testing. The pharmacogenomic report explores the opportunities and challenges associated with PGx research, development of PGx applications, and integration of these applications into clinical practice and public health. To realize the potential of PGx, SACGHS recommendations call for (1) adapting clinical trial designs and data collection tools to enhance assessment of PGx diagnostics and therapies, including sustained data collection to inform clinical practice, payment, and healthcare policy; (2) supporting regulation of PGx products that fosters innovation while ensuring patient safety and improved outcomes; (3) compiling evidence of the clinical utility of PGx testing to obtain coverage and adequate reimbursement of PGx technologies; (4) building a health information technology infrastructure that supports PGx research and PGx-based diagnostic testing, treatment decisions, and surveillance; (5) educating and training clinicians to ensure their competence with PGx technologies; and (6) ensuring equitable patient access to PGx technologies. For the oversight report, SACGHS used a broad interpretation of oversight to include not only federal and state governments and agencies, but also standard-setting organizations, knowledge-generating agencies, public and private sector payers, professional societies, health providers, patients, and consumers. Key SACGHS recommendations include (1) required proficiency testing (PT) for all nonwaived laboratory tests for which PT products are available, (2) Food and Drug Administration involvement to address gaps in the oversight of the clinical validity of genetic tests, (3) mandatory registration of all laboratory tests, (4) creation of a public-private partnership to evaluate the clinical utility and the health impact of genetic tests, and (5) identification of genetic education needs and development of clinical decision support systems. Both SACGHS reports are available at http://www4.od.nih.gov/oba/sacghs.htm.

A Sensitive Functional Assay Reveals Frequent Loss of Genomic Imprinting in Human Placenta. *J. G. Wetmur, L. Lambertini, A. I. Diplas, M.-J. Lee, R. Sperling, J. Chen* Mount Sinai Sch Medicine, New York, NY.

Loss of imprinting (LOI) is the gain of expression from the silent allele of an imprinted gene normally expressed from only one parental copy. LOI has been associated with neurodevelopmental disorders and reproductive abnormalities. The mechanisms of imprinting are varied, with DNA methylation representing only one. We have developed a functional transcriptional assay for LOI that is not limited to a single mechanism of imprinting. The method employs allele-specific PCR analysis of RT-PCR products containing common readout polymorphisms. With this method, we are able to measure LOI at the sensitivity of 1%. The method has been applied to measurement of LOI in human placentas. We found that RNA was stable in placentas stored for more than 1 hour at 4C following delivery. We analyzed a test panel of 26 genes known to be imprinted in the human genome. We found that 18 genes were expressed in placenta. Fourteen of the 18 expressed genes contained common readout polymorphisms in the transcripts with a minor allele frequency >20%. We found that 5 of the 14 genes were not imprinted in placenta. Using the remaining 9 genes, we examined 93 heterozygosities in 27 samples. The range of LOI was 0% - 96%. Among the 93 heterozygosities, we found 23 examples (25%) had LOI >3% and 8 examples (9%) had LOI 1 - 3%. Our results indicate that LOI is common in human placentas. Because LOI in placenta is common, it may be an important new biomarker for influences on prenatal epigenetics. This work was supported by grants NO1 AI50028, U19 AI06231, and P01 ES09584 from the National Institutes of Health and R827039 from the Environmental Protection Administration.

Spondylocheiro Dysplastic Form of the Ehlers-Danlos Syndrome - A Novel Recessive Entity Caused by Mutations in the Zinc Transporter Gene SLC39A13. *C. Giunta[1], NH. Elçioglu[2], B. Albrecht[3], G. Eich[4], C. Bürer-Chambaz[1], AR. Janecke[5], H. Yeowell[6], M. Weis[7], DR. Eyre[7], M. Kraenzlin[8], B. Steinmann[1]* 1) Division of Metabolism and Molecular Pediatrics, University Children's Hospital Zurich, Switzerland; 2) Department of Pediatric Genetics, Marmara University Hospital Istanbul, Turkey; 3) Institute of Human Genetics, University Duisburg-Essen, Germany; 4) Pediatric Radiology, Aarau; 5) Division of Clinical Genetics, Medical University, Innsbruck; 6) Division of Dermatology, Durham, NC; 7) Department of Orthopaedics, Seattle, WA; 8) Division of Endocrinology and Diabetes, Basel.

We present 6 patients from 2 consanguineous families who show EDS-like features and radiological findings of a mild skeletal dysplasia. The EDS-like findings comprise hyperelastic, thin, and bruisable skin; hypermobility of the small joints with a tendency to contractures; protuberant eyes with bluish sclerae; hands with finely wrinkled palms, atrophy of the thenar muscles and tapering fingers. The skeletal dysplasia comprises platyspondyly with moderate short stature, osteopenia, and widened metaphyses. Patients have an increased ratio of total urinary pyridinolines, lysyl pyridinoline / hydroxylysyl pyridinoline, of ~1 as opposed to ~ 6 in EDS VI or ~ 0.2 in controls. Lysyl and prolyl residues of collagens were underhydroxylated despite normal lysyl- and prolyl 4-hydroxylase activities in vitro, and underhydroxylation was a generalized process as shown by MS of the 1(I)- and 2(I)-chain derived peptides of collagen type I and involved at least collagen types I and II. Linkage and sequence analyses identified in all patients a homozygous c.483_491del9 mutation in SLC39A13 that encodes for the zinc transporter SLC39A13. We hypothesize that an increased Zn^{2+} content inside the ER competes with Fe^{2+}, a cofactor which is necessary for hydroxylation of lysyl and prolyl residues, and thus explains the biochemical findings. These data suggest a novel entity which we have designated spondylocheiro dysplastic form of EDS (SCD-EDS) to indicate a generalized skeletal dysplasia involving mainly the spine (spondylo) and striking clinical abnormalities of the hands (cheiro) in addition to the EDS-like features.

Pharmacogenetic analysis of CTLA4 gene polymorphisms and response to tremelimumab in patients with advanced melanoma. *L. Wood[1], J. Richmond[1], F. Gao[2], C. Bulanhagui[3], M. Penny[4]* 1) Pharmacogenomics, Pfizer, Groton, CT; 2) Global Statistics, Pfizer, New London, CT; 3) Oncology, Pfizer, New London, CT; 4) Molecular Medicine, Pfizer, New London, CT.

Background: Tremelimumab is a fully-human, immune-enhancing, monoclonal antibody targeted against CTLA4 that is in clinical development for the treatment of solid tumors. In recognition of the fact that variation in the drug target or other proteins related to tumor immunity may influence a patients response to tremelimumab, pharmacogenomic analysis has been incorporated into the clinical development plan. Methods: Three polymorphisms in the CTLA4 gene, a promoter (-318 C/T), an exon 1 (+49 A/G) and an intergenic (CT60) polymorphism, were genotyped in 268 patients from two tremelimumab trials. All patients had advanced melanoma that was refractory prior to treatment. Tremelimumab was administered at varying dosing regimes. Genotypes were correlated with clinical efficacy endpoints including progressive disease, objective tumor response, and survival at one year, as well as safety endpoints related to diarrhea and severity of diarrhea. Correlations were investigated via Fishers exact tests for contingency tables as well as through logistic regression assessing the interaction effect of sex and genotype. This analysis was powered to detect a relative risk in the >2 range. Results: The two studies were analyzed separately. Allele frequencies were not significantly different from published data, and no significant deviations from Hardy Weinberg Equilibrium were observed ($P < .05$). Data presented shows that no consistent trends were observed between any of the three CTLA4 genotypes and any clinical endpoint tested. Conclusions: No pharmacogenetic effect between the CTLA4 genotypes tested and response to tremelimumab was observed in these initial studies.

Dysregulation of nitric oxide synthesis from endogenous cellular arginine production underlies the complex phenotype in argininosuccinate lyase deficiency and supports metabolite channeling as basis of the Arginine Paradox. *A. Erez[1], Y. Chen[1], N. Bryan[2], J. Marini[3], O. Shchelockhov[1], N. Brunetti-Pierri[1], W. OBrien[1], W. Mitch[4], B. Lee[1,5]* 1) Dept Human Molecular Genetics, Baylor Col Medicine, Houston, TX; 2) Institute of Molecular Medicine The University of Texas - Houston Health Science Center; 3) Department of Pediatrics/ Nutrition USDA/ARS Children's Nutrition Research Center, BCM; 4) Gordon A. Cain Chair in Nephrology Baylor College of Medicine; 5) Howard Hughes Medical Institute.

Argininosuccinic aciduria (ASA) results from enzymatic deficiency of argininosuccinate lyase (ASL), which generates arginine, the precursor of nitric oxide (NO), from ASA. While patients may present with hyperammonemia, their complex natural history reflect other pathophysiologic processes. ASA is a unique genetic model that provides an opportunity to assess the different contributors to NO synthesis and may help explain the basis of the Arginine Paradox in NO biology. We first performed stable isotopic measurement of the flux of arginine to citrulline as a surrogate of whole body NO production. We found that ASA subjects showed significantly decreased NO production. To assess the consequences of ASL deficiency; we generated a hypomorphic mouse model of ASL by introducing a floxed/Neo+ allele. These mice have decreased Asl activity and unlike other models of urea cycle deficiency, they survive weaning exhibiting significant growth restriction, hypertension, liver dysfunction and die around 4 weeks of age. These clinical features could reflect deficiency of NO production. In fact, Asl deficient mice show significantly decreased protein nitrosylation indicating dramatic NO deficiency. These human and mouse genetic data support dysregulation of NO production as a critical pathophysiological process in urea cycle disorders and potentially secondary disorders of ureagenesis, proving that intracellular metabolite channeling of UC intermediates is a major mechanism for directly regulating NO production. From a therapeutic perspective, these data suggest that supplementation of UC intermediates may be more efficacious in a variety of disease processes attributed to NO dysregulation.

Development of genome-wide SNP panel (6.3k) for human identification using tagging approach. *S. Guha[1,2], G. Jianye[2], R. Chakraborty[2]* 1) Division of Molecular Genetics, Department of Pediatrics, Columbia University Medical Center, NY, USA; 2) Center for Genome Information, Department of Environmental Health, University of Cincinnati, OH, USA.

Genome wide panels of Single Nucleotide Polymorphisms (SNPs), studied in four HapMap populations, yielded an wealth of information that are currently used primarily for detecting genes underlying complex disease phenotypes and quantitative phenotypes. Though in principle SNPs offer certain operational advantages over other types of genomic markers (e.g., STRs) for use in human identification, the developed panels of SNP markers for use in DNA forensics are of limited value because collectively they have limited power of discrimination, and do not have genome wide coverage. The main hindrance of developing genome wide panel of SNPs for human identification is the linkage disequilibria (LD) that exist between closely linked SNPs throughout the genome. High LD results in redundancy of genotypic information, and consequently loss in collective power of discrimination. However, the concept of tagged SNPs, employed in disease-gene association studies, can ameliorate this problem. In this computational study with the 500k genome wide dataset of four HapMap populations, we have used this concept that helped in eliminating SNPs that are of high LD with other informative ones. This yielded a panel of 6,354 highly informative SNPs. Being devoid of high LD between them (avg. r^2 0.05); they are collectively highly informative for human identification and relatedness between individuals. Further, this panel constitutes SNPs that are individually most informative i.e., small differences of frequencies of alternative alleles (avg. 0.20) and high heterozygosity (avg. H 0.47) as well as have low levels of allele frequency differences across the HapMap populations (avg. F_{ST} 0.03). The match probabilities of this panel for all four HapMap populations are 0.00. Studies of robustness of their genotypes in forensic samples and surveying their allele frequencies in world wide representative populations should provide a genome wide tagged set of SNPs for wider forensic applications.

Database Indexing for Production MegaBLAST Searches of the Human and Mouse Genomes. *A. A. Schaffer, A. Morgulis, G. Coulouris, Y. Raytselis, T. L. Madden, R. Agarwala* National Center for Biotechnology Information; National Institutes of Health; Department of Health and Human Services; Bethesda, MD 20894 USA.

The BLAST software package for sequence comparison speeds up homology search by preprocessing a query sequence into a lookup table. Numerous research studies have suggested that preprocessing the database instead would give better performance. However, production usage of sequence comparison methods that preprocess the database has been limited to programs such as BLAT and SSAHA that are designed to find matches when query and database subsequences are highly similar. We developed a new version of the MegaBLAST module of BLAST that does the initial phase of finding short seeds for matches by searching a database index. We also developed a program makembindex that preprocesses the database into a data structure for rapid seed searching. We show that the new ``indexed MegaBLAST'' is faster than the ``non-indexed'' version for most practical uses. We show that indexed MegaBLAST is faster than miBLAST, another implementation of BLAST nucleotide searching with a preprocessed database, for most of the 200 queries we tested. Indexed MegaBLAST is now used at NCBI for production nucleotide searches of the human and mouse genomes, handling over 13,000 user queries on typical weekdays. To deploy indexed MegaBLAST as part of NCBI's Web BLAST service, the storage of databases and the queueing mechanism were modified, so that some machines are now dedicated to serving queries for a specific database. The response time for such Web queries is now faster than it was when each computer handled queries for multiple databases. Linux command-line executables for blastn and makembindex, documentation, and some query sets used to carry out the tests we did are available in the directory: ftp://ftp.ncbi.nlm.nih.gov/agarwala/indexed_megablast.

INF- +874 Polymorphism in the first intron of the human INF- gene and kidney allograft outcome. *J. crispim[1,2, 3], I. Wastowski[1], C. Mendes-Júnior[1], C. Bassi[2], E. Castelli[2], R. Costa[4], L. Saber[5], F. Oliveira[3], P. Freire[3], E. Donadi[1,2]* 1) Department of Biochemistry and, University of São Paulo, Ribeirão Preto, SP, Brazil, SP; 2) Division of Clinical Immunology, University of São Paulo, Ribeirão Preto, SP, Brazil; 3) Department of Clinical Analysis; School of Pharmaceutical Sciences, University Federal Rio Grande of Norte (UFRN) Brazil; 4) Department of Pathology, FMRP-USP, University of São Paulo, Ribeirão Preto, Brazil, SPl; 5) Renal Transplant Unity, Clinical Nephrology, FMRP-USP, Brazil.

The aim of this study was to assess the effect of this genetics allelic variation on acute rejection or chronic allograft nephropathy after kidney transplantation. In order to determine a possible correlation between the INF- +874 polymorphism and kidney allograft outcome. After Human Research Ethics Committee Approval. we isolated genomic DNA from 74 patients who had received isolated kidney allografts, and we classified the 74 specimens into two groups: grafts presenting Banff features of rejection (RG) and a non-rejection (NRG) group and compared them with a control group of 163 healthy subjects. The INF- +874 polymorphism was genotyped in all groups. There was no significant difference in allelic and genotype frequencies of INF- + 874 polymorphisms between normal controls and kidney transplant patients. In the rejection group, the homozygous genotype T/T ($p = 0.0118$) was significantly increased in the group with acute rejection compared with the healthy control group. Similarly, considering only patients with chronic allograft nephropathy, the homozygous genotype T/T ($p = 0.0067$) significantly increased in the chronic allograft nephropathy group with compared with the healthy control group. Still, the analysis of the rejection group indicated a significant increased homozygous genotype T/T ($p=0.0061$) when compared with the control group. Homozygous genotype T/T that have been associated with increased level de INF- is associated with a high number of acute rejection and chronic allograft nephropathy episodes after transplantation.

FCG receptor IIB and IIIB polymorphisms and susceptibility to systemic lupus erythematosus and lupus nephritis : A meta-analysis. *Y. Lee, G. Song* Division of Rheumatology, Korea Univeristy, Seoul, Seoul, Korea.

Objective. The aim of this study was to explore whether polymorphisms of the FCG receptors (FCGRs) IIB T/I232 and FCGRIIIB NA1/NA2 confer susceptibility to systemic lupus erythematosus (SLE) and lupus nephritis (LN). Methods. The authors conducted a meta-analysis on associations between the FCGRIIB T/I232 and FCGRIIIB NA1/NA2 polymorphisms and SLE and LN susceptibility as determined using 1) allele contrast, 2) recessive, 3) dominant models, and 4) contrast of homozygotes, using fixed and random effects models. Results. A total of 16 separate comparisons were considered, involving 9 Asian, 6 European, and 1 African population samples, consisting of 2,887 SLE patients and 3,105 controls. Meta-analysis of the FCGRIIB T/I232 polymorphism revealed a significant association between the FCGRB T allele and the risk of developing SLE compared to the FCGRB I allele (OR = 1.207, 95% CI = 1.061 - 1.373, P = 0.004), with no evidence of between-study heterogeneity. In subjects of Asian descent, a significant association was observed between the FCGRIIB T allele and SLE (OR = 1.332, 95% CI 1.138 - 1.558, P < 0.001). However, in Europeans no such association was found. In contrast, no association was found between SLE or LN and the FCGRIIIB NA1/NA2 polymorphism in all subjects, or in European and Asian populations. Conclusions. This meta-analysis demonstrates that the FCGRIIB T/I232 polymorphism confers susceptibility to SLE, especially in Asian-derived populations. In contrast, no association was found between the FCGRIIIB NA1/NA2 polymorphism and susceptibility to SLE or LN in European or Asian populations.

Juvenile glaucoma associated with nail-patella syndrome. *K. Fukai[1], T. Oshimo[1], N. Higashi[2], T. Kitano[3], Y. Imai[4], H. Shintaku[5], M. Ishii[1]* 1) Dept Dermatology, Osaka City Univ, Osaka, Japan; 2) Higashi Dermatologic Clinic, Sakai, Japan; 3) Dept of Pediatric Orthopaedics Surgery, Osaka City General Hospital, Osaka, Japan; 4) Dept of Orthopaedics Surgery, Osaka City Univ, Osaka, Japan; 5) Dept of Pediatrics, Osaka City Univ. Osaka, Japan.

The nail-patella syndrome (NPS, OMIM#161200), also known as hereditary osteoonychodysplasia, is a rare autosomal dominant disorder that is characterized by nail and bone abnormalities and, frequently, renal disease, NPS is caused by a loss of function mutation in the transcription factor IMX1B at 9q34. A 5-year-old girl presented with hypoplastic fingernails from birth. Her thumbnails exhibited severe dysplasia, the index fingernails were ridged longitunianlly, and the thrid and fourth nails exhibited triangle lunules. The creases of the skin overlying the distal interphalangeal joints of the fingers were lost. X-ray examination of the knees and pelvis revealed absence of centers of calcification in the patella and the presence of typical iliac horns. No proteinuria or hematuria was detected. At the age of six, open angle glaucoma developed. This is unusual because most of the reported cases with the association of glaucoma are of the age 40 or older. Heterozygous deletion of two bases (c.368_369delTG) within the coding sequence of exon 3 of LMX1B was found. Unexpectedly, the same mutation was noted in the proband's father, who was thought to be 'normal' by physical examination. This underscores the importance of mutation analysis for the accurate genetic counseling.

The -514C/T polymorphism of hepatic lipase gene among Iranian patients with coronary heart disease. *K. Ghatreh Samani[1], M. Noori[2], M. Rohbani Nobar[3], M. Hashemzadeh Chaleshtory[4], E. Farrokhi[4]* 1) Department of Clinical Chemistry, Tabriz University of Medical Sciences, Tabriz, Iran; 2) Biotechnology Research Center, Tabriz University of Medical Sciences, Tabriz , Iran; 3) Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz , Iran; 4) Cellular and Molecular Research Center , Shahrekord University of Medical Sciences , Shahrekord, Iran.

The T allele of the hepatic lipase (HL) C-514T polymorphism was previously found to be associated with lower plasma HL activity. Here, we examined the association between this polymorphism and plasma HDL-cholesterol concentrations in patients with coronary arteries stenosis. We studied 342 subjects undergoing coronary angiography in two groups of non CAD (n=146) and CAD (n=196) . -514CT polymorphism was determined using polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP). After adjustment for age, smoking ,fasting blood glucose and body mass index, HDL-cholesterol concentrations were significantly higher in men with the C/T&T/T genotype than those with the C/C genotype(mean 38.6 and 34.7 respectively P=0.01). The frequency of T allele in non CAD was 0.136 and 0.226 in female and male respectively and 0.170 and 0.223 for female and male in CAD subjects.There was no difference in T allele frequency in CAD and none CAD groups in male and female (p=0.466 and 0.722 respectively). We concluded that -514CT of LIPC gene have a positive effect on HDL-C concentration especially in male gender .How ever, no difference were determined in frequency of T allele between CAD and normal arteries subjects. Key words: Hepatic lipase gene, HDL-C, Coronary artery stenosis, T allele.

Altered gene-expression in medication-free schizophrenia patients. *T. Rietkerk[1], M. P. M. Boks[1], W. Cahn[1], I. E. C. Sommer[1], R. L. van Ojen[1], S. de Jong[2], C. Schubart[1], R. S. Kahn[1], R. A. Ophoff[2]* 1) Department of Psychiatry and The Rudolf Magnus Institute of Neuroscience, University Medical Centre Utrecht, the Netherlands; 2) Complex Genetics Section, DBG-Dept Medical Genetics and The Rudolf Magnus Institute of Neuroscience, University Medical Centre Utrecht, the Netherlands.

Schizophrenia is a severe psychiatric disorder with a genetic contribution estimated at around 80%. Changes in gene-expression constitute a common mechanism by which genes affect the phenotype. We therefore performed a gene expression study in schizophrenia. RNA samples were taken from peripheral blood cells of schizophrenia and schizoaffective patients, who were free of antipsychotic drugs. Genome-wide microarray analysis was done to compare expression levels between 22 patients and 22 healthy controls, matched on age, sex and substance abuse. In addition we used another sample consisting of 33 healthy controls for further validation. In both case-ctrl analyses we observed significant expression changes in four genes after correcting p-values for the false discovery rate: SPTLC1, MYST3, USP49 and PPARBP. The SPTLC1 gene codes for serine palmitoyl-transferase which is a key enzyme in the biosynthesis of sphingolipids, the main components of myelin sheaths. The protein encoded by MYST3 is also involved in sphingolipid metabolism among other functions. The USP49 protein functions in the ubiquitin cycle and PPARBP is involved in androgen receptor functioning and transcription regulation. These four genes are expressed in brain-tissue as well. We identified genome-wide significant gene-expression changes in schizophrenia patients that are not attributable to medication effects. The finding of altered expression of SPTLC1 and MYST3, involved in sphingolipid metabolism, may reflect aberrant myelin formation in schizophrenia.

Association of I405V and -629C/A polymorphisms of cholesteryl ester transfer protein gene with high-density lipoprotein cholesterol levels in coronary artery disease. *M. Noori[1], K. Ghatreh Samani[2], M. Rohbani Nobar[3], M. Hashemzadeh Chaleshtory[4], E. Farrokhi[4], N. Aslanabadi[2]* 1) Biotechnology Research Cente, Tabriz University of Medical Sciences, Tabriz, Iran; 2) Biotechnology Research Center, Tabriz University of Medical Sciences, Tabriz , Iran; 3) Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz , Iran; 4) Cellular and Molecular Research Center , Shahrekord University of Medical Sciences , Shahrekord , Iran.

Background: Cholesteryl ester transfer protein (CETP) exchanges neutral lipids between lipoproteins. CETP gene is known to have many single nucleotide polymorphisms which have been associated with plasma high density lipoprotein cholesterol (HDL-C) concentrations. The role of CETP in the atherogenic process is still not fully clarified. We studied the association of -629C/A and I405V polymorphism in CETP gene with coronary artery disease (CAD). Methods: We undertook a cross- sectional analysis on two polymorphism of CETP gene among 323 consecutive patients who underwent coronary angiography. Association was analyzed among plasma lipid and lipoproteins, its gene polymorphisms and the finding in coronary angiography. Polymorphisms were determined using polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP). Results: The allele frequencies of -629C/A: A allele was 0.66; and that of I405V: V allele was 0.35. Study of association between I405Vpolymorphisms and plasma lipid and lipoprotein concentrations revealed no significant differences between three genotypes. A genotype based subgroup analysis revealed a significant increase in HDL-C in non-CAD patients with AA and CA genotype. There was significance difference with A allele frequency in CAD and non-CAD group. Conclusions: In the present study there was no change in HDL-C between the I405V genotypes and CAD prevalence was not significantly different among in these three genotypes. AA genotype of -629 CA polymorphism in promoter of CETP gene increased HDL but this genotype maybe associated with higher risk of CAD than CC genotype. Keywords: CETP gene, I405V, HDL cholesterol,-629C/A.

The international multidisciplinary Community Genetics Network. *L. P. ten Kate[1], L. Henneman[2]* 1) Dept Clinical Genetics, EMGO-Institute, VU University Medical Center, Amsterdam, The Netherlands; 2) Dept Public and Occupational Health, EMGO-Institute, VU University Medical Center, Amsterdam, The Netherlands.

The International Multidisciplinary Community Genetics Network is a non-commercial E-mail network aiming to facilitate communication among those working in the field of community genetics. This includes researchers, health professionals and others interested in genetic screening, genetic education, access and quality of genetic services or preconception care, genetics in primary care, genetic registries, genetics for low-income countries and other disadvantaged populations, public consultation and epidemiologic, economic, psychosocial, ethical and legal issues. The core activity is a monthly newsletter with a list of references to recent scientific papers of members and a continuously updated list of upcoming meetings. This allows a rapid spread of information towards other members, which is advantageous to the authors of papers or organizers of meetings, but also to readers who want to stay tuned on what others are doing. In addition members may present information on new activities in the newsletter, and publish calls for information (e.g. about validated research questionnaires or participants for a particular study). The Network was launched May 2007. One year later (June 1, 2008) the Network has 343 members in 51 countries worldwide, growing at a pace of 20-30 new members each month. One third of the membership comes from the United States (88 members) and Canada (26 members). Forty five percent of the members are from Europe and twelve percent from Asia. Updated numbers and further details will be presented at the ASHG annual meeting in Philadelphia. Those who want to have more information or want to become a member and obtain the newsletter, should send an E-mail to commgennet@gmail.com.

Samples in, data out? *B. Knoppers* Dept CRDP, Univ de Montreal, Montreal, PQ, Canada.

In the explosion of large, genomic or disease population studies seeking to pool or federate datasets, unforeseen issues arise. Prospective studies can map out future interoperability through broad consent and recontact mechanisms but the retrospective use of already collected samples and data may encounter severe limitations due to ELSI constraints. The 2008 passage of the Genetic Information Non-Discrimination Act (GINA) in the United States illustrates a legislative approach to the issue of potential discrimination in health insurance. Other countries have adopted this genetic-specific legislative model but in the context of biobanks. A second approach has been to subsume the issues of consent and confidentiality in genomic research under the personal data protection umbrella. Sufficient interpretative leeway may also be found via human rights protections as sufficiently flexible for the framing of sample and data access and use issues as they arise. Perhaps a medical-model would provide an avenue closer to the original intent of the sample-sources however. This would hold true across the spectrum going from population genomic studies, to molecular epidemiology, to hopefully, the translation into genomic medicine for common chronic diseases. An examination of examples from various international (GAIN; WTCC; ENGAGE) and national genomic studies (UK Biobank; CARTaGENE; Japan Biobank) will serve to test these models. If samples are voluntarily provided to be used in research, why are there barriers to access resulting data?

Genome-wide oligonucleotide Array CGH for etiological diagnosis of mental retardation and autism spectrum disorders: a multi-center experience on 1,551 clinical cases. *Y. Fan[1], B. Xiang[1], B. Wu[2], P. Li[3], M. Li[4], T. Chen[5], H. Zhu[1], Y. Shen[2], K. Lu[3], M. Mahoney[3], M. Seashore[3], A. Bale[3], J. McGrath[3], H. Andersson[4], T. Narumanchi[4], X. Hu[4], Y. Wang[5], J. Martinez[5], U of Miami Clin Genet Group* 1) University of Miami Miller School of Medcine, Miami, FL; 2) Harvard Medical School, Boston, MA; 3) Yale University School of Medicine, New Haven, CT; 4) Tulane University School of Medicine, New Orleans, LA; 5) College of Medicine, University of South Alabama, Mobile, AL.

Mental retardation (MR) occurs in ~3% of the general population whereas about 1/150 children have an autism spectrum disorder (ASD). ~70% of autism patients also have MR. Recent aCGH studies have revealed pathogenic gene copy number variations (CNVs) in >10% of patients with MR and ASDs in addition to chromosomal abnormalities detectable by conventional karyotyping. We have collected aCGH results on 1,551 cases from 5 cytogenetics laboratories where Agilent 44K platform has been used as a clinical test. Among these cases, 1,441 had unexplained MR and 110 had a diagnosis of autism or ASD. Pathogenic CNVs were detected in 6.8% ~14% of cases with MR by 5 different labs with an overall detection rate of 11.6%. In ASD patients, the overall detection rate for pathogeinc CNVs was 10.9% but varied from 3.2% to 16.7% in 3 labs. It is apparent that pathogenic CNVs occur with a similar frequency in patients with MR and ASD. Our results have demonstrated that a genome-wide array CGH such as the 44K is a powerful diagnostic tool for patients with unexplained MR or autism. With the collective data, we are able to address the important issues involved in clinical use of aCGH, such as the criteria of interpretation of CNVs, cutoff range, confirmation, and cost-effectiveness of test. Further comparison and characterization of the recurrent novel pathogenic CNVs identified in our study will lead to discovery of new clinical syndromes and disease genes.(U of Miami Clin Genet Group: P Jayakar, D Barbouth, S Sacharow, K Wierenga, V Carver, A Morales, LJ Elsas).

Multiple mechanisms of RET kinase domain mutations in Hirschsprung disease: A structure-function study. *B. D. Hyndman, T. S. Gujral, J. R. Krieger, L. M. Mulligan* Division of Cancer Biology and Genetics , Cancer Research Institute, Queen's University, Kingston, Ontario, Canada.

The proto-oncogene RET encodes a receptor-tyrosine kinase important for kidney morphogenesis and maturation of several neural crest-derived cell lineages of the peripheral nervous system. Mutations of the RET gene have been reported in Hirschsprung disease (HSCR), a congenital abnormality associated with the absence of enteric ganglia in the intestinal tract, and are thought to result in loss of RET function. With the recent publication of the three-dimensional structures of inactive and activated RET we set out to characterize the molecular mechanisms of HSCR mutations and how they can lead to receptor dysfunction. We used our RET kinase three-dimensional models to predict structural changes and downstream functional consequences of 23 HSCR-associated missense mutations located within the RET tyrosine kinase domain. A representative subset of these mutants were evaluated in cell based assays to examine the functional effects of these mutations on RET. Briefly, assays were performed to determine the effects of the mutations on RET protein localization to the cell surface, RET autophosphorylation following ligand binding as well as on its ability to interact with adaptor proteins and activate downstream signaling pathways. We also evaluated the ability of the various RET mutants to induce changes in cell growth and apoptosis. We show that HSCR mutations in the tyrosine kinase domain vary in the degree of RET impairment, and that not all mutations have the same effects on RET-mediated processes. Our data indicate that HSCR mutations of RET can result in dysfunction of the RET protein by several mechanisms, depending on both the position, and type of mutation. Importantly, these effects are not limited to kinase inactivation.

Caucasian prostate cancer patients and VEGF gene haplotypes. *K. Yanamandra[1], M. Ankem[4], D. Napper[1], P. B. Boggs[2], H. Chen[1], S. A. Ursin[1], G. Mills[3], J. A. Bocchini Jr.[1], R. Dhanireddy[5]* 1) Dept Pediatrics, LSU Medical Ctr, Shreveport, LA; 2) Allergy Clinic, Shreveport, LA; 3) Feist-Weiller cancer center, Shreveport, LA; 4) Dept Urology, Robert Wood Johnson Medical School, New Brunswick, NJ; 5) Dept Pediatrics, UT Health Sciences Center, Memphis, TN.

Prostate cancer is the second leading cause of death among male cancer patients second only to lung cancer. A host of genetic factors influence the tumorigenesis and cancer etiology, especially the combination of low-penetrance gene polymorphisms (1,2) Because angiogenesis is a major feature and is an essential process in the development, growth and metastasis of malignant tumors (3-5), in the present investigation we began to study the role of Vascular Endothelial Growth Factor (VEGF) gene polymorphisms in the etiology of prostate cancer. Our study population was Caucasian patients and controls. We have studied the influence of VEGF gene SNPs both in the promoter and in the coding regions, in thirteen prostate cancer patients and ninety five controls. The genetic polymorphism studies were carried out by microplate PCR RFLP and allele specific PCR genotyping methods. Among the genetic markers studied, mutant genotypes in the promoter and coding region of VEGF gene showed a significant difference in their frequencies (odds ratio(OR) 9.5, p value 0 for -460T, OR 2.7, p value 0.02 for -1154G). Also, the haplotype data analysis revealed that the VEGF-460T/VEGF-1154G haplotype frequency was significantly higher among the prostate cancer patients compared to the controls (OR 2.4, p value 0). Based on our experimental data we conclude that the VEGF-460T/VEGF-1154G haplotype was a significant risk factor in the etiology of prostate tumorigenesis among Caucasians. References: 1. Shields PG, et al., J Clin Oncol 2000;18:2309-2315. 2. Mohrenweiser HW, et al., Mutat Res 1998;400:15-24. 3. Folkman J, et al., Nature 1989;339:58-61. 4. Shi YP, et al., Biochem Biophys Res Commun. 1999;254:480-483. 5. Yancopoulos GD, et al., Nature 2000;407:242-248.

Pseudogene-derived IKBKG Gene Mutation in Incontinentia Pigmenti. *NC. Lee[1], CH. Huang[2], WL. Hwu[1], YH. Chien[1], TM. Ko[2]* 1) Dept Medical Genetics & Pediatrics, National Taiwan Univ Hosp, Taipei, Taiwan; 2) Ko's Obstetrics and Gynecology Clinic, Taipei, Taiwan.

Background Incontinentia pigmenti (IP; MIM #308300) is a rare X-linked-dominant disease involving abnormalities in the skin, hair, teeth, nail, eyes, and the central nervous system. IKBKG (NEMO; GeneID:8517) is causal gene, and a recurrent deletion of exon 4-10 accounts for 60-90% of the detected mutations. Recently, a pseudogene IKBKGP (NEMO; GeneID: 246210) containing exon 3-10 of IKBKG in an opposite direction was located close to IKBKG. Materials and Methods Thirty-two blood samples and 9 prenatal samples from 20 families were analyzed for IKBKG gene mutation. Long-range PCR was performed to detect the 4-10 deletion. For samples negative for the exon 4-10 deletion, direct sequencing of each exon was performed. Pseudogene-specific primers were used to amplify the IKBKGP pseudogene followed by direct sequencing. Results Mutations of the IKBKG gene were identified in 11 families (13 patients and 2 fetuses). Therefore, the detection rate was 55% (11 in 20 families). Among the 11 families, 7 had the exon 4-10 deletion (63.6% of the detected mutations). Besides, 1 family had a novel mutation c.1110insTTdelC (p.Ala371CysfsX24). While another 3 families had a novel mutation c.520-523dupCAGG (p.Ala174GlnfsX15) in exon 5. In Family 1, the c.520-523dupCAGG mutation of the IKBKG gene was only found in the patient (heterozygous), but heterozygous c.520-523dupCAGG mutation in IKBKGP was found in the patient, the mother, the youngest aunt, and the grandmother, indicating this mutation was already resident in the pseudogene in the unaffected mother and relatives. Discussion We demonstrated that the c.520-523dupCAGG mutation in the IKBKGP gene was carried in several individuals in one family including the mother of the patient. In the patients, the mutation was resided at both the true gene and the pseudogene. Therefore it is likely that a recombination occurred and the true gene was converted by the pseudogene. This is the first evidence for the involvement of the pseudogene for small mutations in IP.

Spectrum of Development in Aicardi Syndrome. *B. L. Kroner[1], W. D. Gaillard[2]* 1) Statistics and Epidemiology, RTI International, Rockville, MD; 2) Neurosciences, Children's National Medical Center, Washington, DC.

Purpose: Aicardi syndrome (AIC) is a rare neurological disorder clinically defined by agenesis of the corpus callosum (ACC), chorioretinal lacunae and infantile spasms. Other features include brain, eye and skeletal abnormalities and global developmental disabilities. AIC is presumed to be X-linked but no gene has been identified. Few reports identify children who have unassisted ambulation, speech and self-help skills. We sought to describe the spectrum of developmental in AIC and to identify early predictors of high functioning cases. Methods: 95 cases of AIC, at least 2 yrs of age, were identified from a parent survey which included questions on signs/symptoms related to the diagnosis of AIC, seizure history and treatment, developmental progress and autistic-related behaviors. Results: Median age of child at the time of survey was 10 yrs (range 2-30 yrs). Daily seizures occurred in 53 (58%). 46 (48%) had at least one autistic-related behavior. 19% had a chronic or permanent loss of skills due mostly to seizures (61%), medications (35%), and surgery or illness (25%). 51 cases (59%) had a developmental age (DA) <12 months. Of these, mean age at seizure onset was 2 months, 28% have partial ACC, 63% have vertebral anomolies, 27% have microphthalmia, 61% eats by mouth, and 10% recognize a familiar adult. 30 cases (34%) had a DA >24 months. Of these, mean age at seizure onset was 3.2 months, 41% have partial ACC, 23% have vertebral anomolies, 7% have microphthalmia, 100% eats by mouth, and 93% recognize a familiar adult. Example of skills accomplished in the 30 with DA >24 months include: walk alone (83%), toilet trained (57%), dresses self (27%), use utensil to eat (88%), count 5 objects (50%), read words (23%), understand conversation (77%) and speak >20 words (67%). Conclusions: Developmental disabilities are common in AIC but a higher proportion than previously reported have unassisted ambulation, independent life skills, verbal communication and demonstrated academic learning. Possible early predictors of higher functioning status include age at seizure onset >3 months; partial ACC; absence of secondary AIC features, and early cognitive awareness.

Which first? Array Comparative Genomic Hybridization or G-banded karyotype. *F. Li[1], E. Lisi[1], J. Hoover-Fong[1], V. Kottoor[1], J. Chinsky[1], D. Batista[2,3]* 1) McKusick-Nathans Institute od Genetic Medicine, Johns Hopkins University, Baltimore, MD; 2) Department of Pathology, Johns Hopkins University, Baltimore, MD; 3) Cytogenetics Laboratory, Kennedy Krieger Institute, Baltimore, MD.

We present two patients to discuss the implications of a-CGH and the two-step approach currently used in cytogenetics. Patient 1 was an 11 year-old girl mildly dysmorphic with gross developmental delay. G-banding showed an abnormality in chromosome 11 initially interpreted as a deletion within band p13: del(11)(p13p13). A-CGH showed no copy number changes in chromosome 11 or others. The karyotype was reevaluated as a possible inversion: inv(11)(p11.3p13). Patient 2 was a newborn girl dysmorphic with severe cardiac defect. 20 G-banded cultured peripheral blood cells showed normal karyotype. A-CGH was suggestive of mosaic trisomy 14. FISH showed trisomy 14 in 6% of cultured vs. 21% of uncultured peripheral blood cells. This discrepancy is likely due to different cell populations studied; all types of nucleated cells are analyzed in the uncultured specimen, whilst selective growth advantage of karyotypically normal lymphocytes might exist in the cultured specimen. FISH of uncultured cells should more accurately represent the level of mosaicism in the patients blood and be comparable to the a-CGH result. Our current two-step diagnostic approach is to first perform karyotype analysis. If normal, a-CGH is used to evaluate smaller genomic changes. These two patients were examples of a-CGH providing information beyond conventional cytogenetics. In patient 1, the chromosome 11 abnormality was misinterpreted by initial G-banding. However, if only array had been performed, the probable inverted 11 would be missed while the inversion could be related to the patients phenotype. In patient 2, a-CGH detected mosaic trisomy 14 that was also missed by the initial G-banding. Should we reverse the sequence of the tests or perhaps routinely perform them together? A-CGH and chromosome G-banding are two independent techniques providing complementary information. Simultaneous execution of these complementary tests would reduce tests turn around time and provide the most complete information possible to the patient.

Concurrence of Muir-Torre and Turcot Variants of Hereditary Nonpolyposis Colorectal Cancer (HNPCC). *C. A. Griffin, J. E. Axilbund, C. D. Gocke, A. Piurek, F. M. Giardiello, K. M. Murphy* Depts of Pathology, Oncology, and Medicine, Johns Hopkins Univ, Baltimore, MD.

HNPCC is a cancer-predisposition syndrome causing primarily colorectal (CRC) and/or endometrial cancer at an early age. Recognized variants are Muir-Torre syndrome (MTS), in which sebaceous skin tumors are identified, and Turcot syndrome type I, in which astrocytomas and glioblastomas occur. To our knowledge, the presence of manifestations of both variants in a family with a documented mutation in a mismatch repair gene has been reported only twice. We have recently seen 3 families with mismatch repair gene mutations and both sebaceous tumors and brain tumors. The first is a family that met Amsterdam-I criteria in which the proband presented with CRC at age 41, sebaceous adenoma (SA) and sebaceous carcinoma (SC) at age 44, and mixed anaplastic astrocytoma (MAA) at age 45. Microsatellite instability (MSI) was observed in both the SA and MAA. He had a brother and father with colon cancer. Deletion of exons 8-15 in MSH2 was identified in the father. The second family did not meet Amsterdam-I or II criteria, but included an individual with mucinous CRC of the cecum at age 46, SC at age 55, MAA at age 57, and deletion of exons 1-6 in MSH2. His mother had uterine cancer at age 45 and stomach or CRC at age 55, and his brother, also mutation positive, had a son with glioblastoma at age 23. The third family includes an individual with early CRC, a reference to sebaceous adenoma, and a documented IVS9+1G>A MLH1 mutation; there is a paternal history of glioblastoma, multiple individuals with early onset CRC and several cases of small intestine and uterine cancers. Medical record confirmation of the skin lesion and brain tumor is pending. If verified, this will be the first example of concurrent MTS and Turcot syndrome associated with a MLH1 mutation. These families confirm the coexistence of both MTS and Turcot variants in kindreds with HNPCC and suggest that the manifestations of both sebaceous skin lesions and astrocytic brain tumors in HNPCC may not be as rare as previously thought.

***TCOF1* presents differential allelic expression in lymphocytes: a potential mechanism to explain clinical variability in Treacher Collins syndrome?** *C. Masotti[1], D. O. Vidal[2], A. Splendore[1], J. E. de Souza[3], R. Moura[2], F. Cavalher[2], T. Felix[4], N. Alonso[5], A. A. Camargo[2], M. R. S. Passos-Bueno[1]* 1) Instituto de Biociencias, Universidade de Sao Paulo, SP, Brazil; 2) Ludwig Institute for Cancer Research, Sao Paulo Branch, Hospital Alemao Oswaldo Cruz, Brazil; 3) Pos-Graduaçao em Bioinformatica, USP, Brazil; 4) Departamento de Genetica Medica, Hospital de Clinicas de Porto Alegre, UFRGS, Brazil; 5) Departamento de Cirurgia Plastica, Hospital das Clinicas da Faculdade de Medicina, USP, Brazil.

Treacher Collins syndrome (TCS) is an autosomal dominant craniofacial disorder caused by null mutations in *TCOF1* gene, which are predicted to lead to mRNA degradation by nonsense mediated mRNA decay (NMD). Haploinsufficiency of the gene product (treacle) during embryonic development is the supposed molecular mechanism underlying TCS. Among TCS patients with pathogenic mutations, no genotype-phenotype correlation has been observed so far and we have hypothesized that clinical variability may be associated to variation in gene expression levels of the wild-type allele. In the present report we investigated if *TCOF1* transcript levels in lymphocytes were associated to phenotypic variation in TCS. We detected a wide variation of *TCOF1* expression among normal and affected individuals and found a significant difference in *TCOF1* mRNA levels between these two groups. Although we herein demonstrate for the first time that patients' mature cells do not have the same amount of *TCOF1* transcripts as normal individuals, we did not observe any association between transcript levels and severity of the phenotype. We also investigated the methylation status of *TCOF1* promoter's CpG island from normal and TCS affected individuals. The absence of methylated cytosines suggested that this regulatory mechanism is not responsible for individual *TCOF1* expression variation. However, we detected differential expression of *TCOF1* alleles in lymphocytes from patients and controls: some individuals can have one allele with extreme skewed expression, while others can have both alleles equally expressed. We discuss the possibility of association of this mechanism to the remarkable TCS phenotype variation.

Mutation scanning novel transcripts for the CMTX3 gene mutation. *M. Brewer[1,2], G. Nicholson[1,2,3], P. Polly[4,5], M. Kennerson[1,2,3]* 1) Northcott Neuroscience Lab, ANZAC Research Institute, Sydney, NSW, Australia; 2) Faculty of Medicine, University of Sydney, Sydney, NSW, Australia; 3) Molecular Medicine Laboratory, Concord Hospital, Sydney, NSW, Australia; 4) Cancer Pharmacology Unit, Concord Hospital, Sydney, NSW, Australia; 5) Department of Pathology, University of New South Wales, Sydney, NSW, Australia.

X-linked Charcot-Marie-Tooth (CMTX) disease is a common inherited degenerative disorder of the peripheral nerve. CMTX3 is one of five CMTX loci. Our overall aim is to identify the gene mutation causing CMTX3 disease. CMTX3 was first mapped to Xq26-q28 in 1991 [1]. Our laboratory mapped and refined the locus to a 2.5 Mb region on chromosome Xq26.3-q27.1 [2, 3]. The CMTX3 region contains 12 annotated genes (UCSC Genome Browser, Build 36.1). Exclusion of the coding region, splice elements and untranslated region (UTR) of these genes for pathogenic mutations along with expression of partial uncharacterised transcripts in the interval suggests that the disease could be caused by a novel gene. Bioinformatic analyses identified 11 UniGene expressed sequence tag (EST) clusters and 23 single ESTs in the region. Expression of ESTs in neural specific tissue libraries, fetal brain, spinal cord and skeletal muscle, prioritised ESTs to be characterised and screened. Full-length transcripts of ESTs were constructed using 5- and 3-Rapid Amplification of cDNA Ends (RACE). Characterised transcript exons have been routinely screened for the pathogenic mutation using High Resolution Melt analysis. 1. Ionasescu et al. Am J Hum Genet (1991) 48, 1075-1083. 2. Huttner et al. Neurology (2006) 67, 2016-21 3. Brewer et al. Neurogenetics (2008) DOI: 10.1007/s10048-008-0126-4.

Penetrance of *CARD15* genetic variants in the general population: inflammatory bowel disease, biochemical markers and meta-analyses. *S. Yazdanyar[1], P. R. Kamstrup[1], M. Weischer[1], A. Tybjærg-Hansen[2], B. G. Nordestgaard[1]* 1) Department of Clinical Biochem, Herlev Hospital, Copenhagen University Hospital , Herlev, Denmark; 2) Department of Clinical Biochemistry, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark.

Background: In case-control studies of Europeans, Arg702Trp, Gly908Arg, and Leu1007fsincC in the *CARD15* gene associate with the inflammatory bowel disease Crohns disease, but not with ulcerative colitis; however, the penetrance in the general population is unknown. We hypothesized that *CARD15* genetic variants influence risk of inflammatory bowel disease and levels of inflammatory biochemical markers in the general population. In addition, we performed meta-analyses of previous case-control studies. **Methods:** From the general population, we examined 10,597 participants from The Copenhagen City Heart Study, and 32,999 participants from The Copenhagen General Population Study. A total of 162 participants were diagnosed with Crohns disease and 376 with ulcerative colitis from 1976 through July 2007. Meta-analyses included 25 studies for Crohns disease and 14 studies for ulcerative colitis. **Results:** In the combined general population studies, 89% were non-carriers, 11% heterozygotes, and 0.4% compound heterozygtes or homozygotes. For Crohns disease, odds ratios for *CARD15* heterozygotes were 1.2(95% confidence interval 0.8-1.9) and 3.3(0.8-13.6) for compound heterozygotes and homozygotes combined versus non-carriers; corresponding values for ulcerative colitis were 1.1(0.8-1.5) and 0.7(0.1-5.1). Genotype did not influence levels of inflammatory biochemical markers. In the meta-analysis of case-control studies of Crohns disease, odds ratios for heterozygotes were 3.2(2.8-3.8) and for compound heterozygotes and homozygotes combined 10.2(6.4-16.4) versus non-carriers; corresponding values for ulcerative colitis were 1.2(0.9-1.5) and 1.5(0.6-4.0). **Conclusions:** In meta-analyses, *CARD15* genetic variants associate with increased risk of Crohns disease in case-control studies of Europeans; however, the penetrance of these variants is most likely low in the European general population.

Localisation of a fifth gene involved in Autosomal Dominant Hypercholesterolemia. *M. MARDUEL[1], A. MARQUES[1], M. ABIFADEL[1,2], J. BONNEAU[1], M. DEVILLERS[1], D. ERLICH[1], A. MUNNICH[1], J.-P. RABES[1,3], C. BOILEAU[1,3], M. VARRET[1]* 1) Hopital Necker, INSERM U781, Université PARIS Descartes, France; 2) Faculté de Pharmacie, Université Saint-Joseph, Beirut, Lebanon; 3) Laboratoire de Biochimie et de Génétique Moléculaire, CHU Ambroise Paré (AP-HP & Université Versailles-Saint-Quentin-en-Yvelines), Boulogne, France.

Autosomal Dominant Hypercholesterolemia (ADH) is a major risk factor for atherosclerosis, witch cardiovascular complications are the most important cause of morbidity and mortality in industrial country. Genetic factors involved in cholesterol homeostasy are multiple, but identification of genes linked to ADH have contributed to a best understanding of cholesterol metabolism. ADH was initially associated to mutations in 2 genes : LDLR and APOB. Our team has shown that defects in at least 2 other genes (HCHOLA3 and HCHOLA4) are implicated in the disease. We identified HCHOLA3 as PCSK9 (proprotein convertase subtilisin/kexin type 9) and located HCHOLA4 at 16q22.1 in a 5.31 cM interval. Through the ADH French Research Network, we collected genetic material from a large french pedigree (35 samples : 15 affected, 16 normocholesterolemics and 4 spouses). Linkage and sequencing analyses in this family excluded the involvement of the LDLR, APOB and PCSK9 genes. Furthermore, the study of 6 microsatellite markers spanning the HCHOLA4 interval clearly excluded linkage to this locus. These results demonstrate the existence of a HCHOLA5 gene. To evaluate the power of the family for linkage, simulations were carried out. Average and maximum lod score were 2.1 and 4.0 indicating that the statistically-significant threshold of 3 could be reached in this single family. The genomewide scan for 21 individuals was realized with 10k affimetrix arrays. Linkage analyses, using parameters compatible with ADH, allowed us to identify a single locus of 0.9 cM and 939.3 kb (maximum LS = 3.5) including 35 genes. Some of them encode proteins of the cholesterol metabolism and are currently analysed. Identification of HCHOLA5 could reveal a new aspect of cholesterol metabolism, and may lead to the development of new preventive method and/or better aimed drugs.

Constitutional MLH1 promoter methylation as a cause of MLH1 inactivation in patients with early-onset colorectal cancer. *Q. Wang[1, 2], J. Auclair[2], C. Lasset[3], V. Bonadona[3], F. Desseigne[4], S. Giraud[1], J.-C. Saurin[5], M.-O. Joly[6], A. Puisieux[1,2]* 1) Plate-forme Mixte de Génétique Constitutionnelle des Cancers Fréquents, Centre Léon BérardHospices Civils de Lyon, Lyon, France;; 2) Laboratoire de recherche translationnelle, Centre Léon Bérard, Lyon, France; 3) Dept d'Epidermiologie et Génétique, Centre Léon Bérard, Lyon, France; 4) Dept Médecine, Centre Léon Bérard, Lyon, France; 5) Service d'Hépatogastroenterologie, Centre Hospitalier Lyon Sud, Lyon, France; 6) Laboratoire Central dAnatomie et de Cytologie Pathologiques, Hôpital Edouard Herriot,Lyon, France.

Inactivation of the DNA mismatch repair system (MMR) is responsible for a common cancer syndrome, HNPCC, as well as a proportion of sporadic colon cancers. Recently, germline methylation of MLH1 and MSH2 promoters has been demonstrated in putative HNPCC patients, as a mechanism leading to gene inactivation. However, the inheritance of such epigenetic event remains questionable. This leads us to hypothesize that epimutation is one of the causes involved more specifically in young sporadic colon cancer patients because 1) it results in germline monoallelic MMR gene silencing, and consequently, an increased susceptibility and 2) it does not appear to be stably transmitted to offspring. Thus a limited or non-existent family history may be expected. We analyzed 95 sporadic colon cancer patients diagnosed before the age of 50 years using methylation-specific PCR (MCP) combined with sequencing of bisulfite-treated DNA fragments. A total of 4 cases with germline MLH1 promoter epimutation were identified. Interestingly, one case displayed a MSS phenotype with normal MLH1 immunostaining. We also observed discordant results between the sequencing approach and MSP methods. Our results suggest that germline epimutation of MLH1 promoter is responsible for a small proportion of sporadic early-onset colon cancers (4%) and, currently used pre-selection and detection conditions need to be improved to increase the sensitivity and specificity.

Genome wide association study and follow-up analysis of visceral and subcutaneous abdominal fat in Hispanics: the IRAS Family Study. *J. Norris[1], C. Langefeld[2], M. Talbert[2], M. Wing[2], T. Haritunians[3], T. Fingerlin[1], A. Hanley[4], K. Taylor[3], S. Haffner[5], J. Rotter[3], D. Bowden[2], L. Wagenknecht[2]* 1) Colorado School of Public Health, Denver, CO; 2) Wake Forest University, Winston-Salem, NC; 3) Burns and Allen Cedars-Sinai Research Institute, Los Angeles, CA; 4) University of Toronto, Toronto, ON, Canada; 5) University of Texas Health Sciences Center at San Antonio, San Antonio, TX.

Purpose: To identify candidate genes and loci associated with computed tomography (CT)-derived measures of adiposity in Hispanic participants from the IRAS Family Study. Methods: In 1190 Hispanic individuals from 92 families from the San Luis Valley, CO and San Antonio, Texas, we measured CT-derived visceral adipose tissue (VAT), subcutaneous adipose tissue (SAT), visceral: subcutaneous ratio (VSR), and waist/hip ratio (WHR). A pilot genome-wide association study (GWAS) was carried out with 318K single nucleotide polymorphisms (SNPs) in 229 individuals from the San Antonio site. SNPs were analyzed for association with VAT, SAT, VSR and WHR, and those with evidence of association to these phenotypes were genotyped in the entire set of Hispanic samples (n = 1190), adjusting for admixture using a principal components analysis of 80 ancestry informative markers (AIMs). Results: Several SNPs were strongly associated in the pilot GWAS ($p<1x10^{-5}$) and were confirmed to be significantly associated in the follow-up (FUP) analysis of the entire Hispanic sample, including: rs7543757 ($p_{GWAS}=4.6x10^{-6}$; $p_{FUP}= 0.009$) for VAT; rs1377445, ($p_{GWAS}=7.2x10^{-6}$; $p_{FUP}= 0.0038$), rs4754373 ($p_{GWAS}=9.5x10^{-7}$; $p_{FUP}=2.7x10^{-5}$), and rs11212913 ($p_{GWAS}=6.5x10^{-6}$; $p_{FUP}=0.00035$) for SAT, and rs4541696 ($p_{GWAS}=6.1x10^{-6}$; $p_{FUP}=0.0036$), rs371004 ($p_{GWAS}=3.5x10^{-6}$; $p_{FUP}=0.0007$), rs4134351 ($p_{GWAS}=1.6x10^{-6}$; $p_{FUP}=0.0002$) for VSR. While none of the aforementioned SNPs are in genes, several SNPs in genic regions were associated at slightly larger GWAS p-values. Conclusions: Several candidate loci have been identified that are strongly associated with VAT, SAT and VSR in Hispanic Americans in a two-stage genetic association study.

Association of complement factor H, LOC387715, HTRA1 and apoE polymorphisms with age-related macular degeneration in Hungary. *I. Balogh[1], G. Losonczy[2], A. Fekete[1], Z. Voko[3], L. Takacs[2], I. Kaldi[4], E. Ajzner[5], E. Dzsudzsak[1], V. Nagy[2], M. Kasza[2], A. Vajas[2], A. Berta[2]* 1) Clinical Biochemistry, University of Debrecen, Debrecen, Hungary; 2) Department of Ophthalmology, University of Debrecen, Debrecen, Hungary; 3) Department of Preventive Medicine, University of Debrecen, Debrecen, Hungary; 4) Department of Ophthalmology, Kenézy Gyula County Hospital, Debrecen, Hungary; 5) Central Laboratory, Josa Andras County Hospital, Nyiregyhaza, Hungary.

Age-related macular degeneration (AMD) is a leading cause of irreversible central vision loss in the elderly worldwide. The multifactorial disease has a strong genetic component. The goal of this study was to establish the frequency of Tyr402His polymorphism of the CFH gene, Ser69Ala polymorphism at LOC387715, rs11200638 polymorphism of the HTRA1 gene and different ApoE alleles in Hungarian patients with AMD and to determine disease risk conferred by these factors. Materials and methods: In a case-control study, we performed clinical and molecular genetic examination of 105 AMD patients and 95 unrelated healthy controls. According to disease severity, 48 patients (46%) were assigned to the early and 57 patients (54%) to the late AMD subgroup. Results: Carriers of at least one CFH, LOC387715 or HTRA1 risk allele were at 1.8-fold (95%CI:1.0-3.3), 2.0-fold (95%CI:1.1-3.6) or 2.2-fold (95%CI:1.2-4.0) increased disease risk, respectively. In the early AMD subgroup, homozygous CFH, LOC387715 or HTRA1 polymorphisms conferred 4.9-fold (95%CI: 1.7-14.2), 7.4-fold (95%CI: 2.1-26.2) or 10.1-fold (95%CI: 2.5-40.8) greater likelihood of disease, respectively. In the late AMD subgroup, carriers of two CFH, LOC387715 or HTRA1 risk alleles were at 10.7-fold (95%CI: 3.7-31.0), 11.3-fold (95%CI: 3.2-40.4) or 13.5-fold (95%CI: 3.3-55.4) greater disease risk, respectively. We found no association between ApoE alleles and AMD in our groups. Conclusions: The CFH, LOC387715 and HTRA1 polymorphisms strongly associate to the development of AMD in Hungary. The association is particularly strong when homozygous risk alleles are present and in late stages of the disease.

FISH analysis of acute graft-versus-host disease in living-related liver transplant with one-way donor-recipient HLA-matching. *K. Kanehira[1], D. L. Riegert-Johnson[2], D. Chen[1], S. D. Grinnell[1], G. V. N. Velagaleti[1]* 1) Department of Lab Medicine & Pathology, Mayo Clinic, Rochester, MN; 2) Department of Internal Medicne, Mayo Clinic, Rochester, MN.

Acute graft-versus-host disease (GVHD) is an uncommon complication following liver transplant but the outcome is often fatal. Clinical diagnosis of GVHD is difficult because the clinical presentations, fever, skin rash, diarrhea and bone marrow failure, are non-specific. We describe a severe GVHD case in a female patient who underwent living-related liver transplant from her son for primary biliary cirrhosis and secondary cholangiocarcinoma. The HLA typing of the donor was homozygous at all loci. The recipients HLA was haploidentical to the donor, forming a donor-dominant one-way HLA match. Four weeks after transplantation, the patient developed pancytopenia which required treatment with granulocyte-colony stimulating factor. Bone marrow aspirate revealed severely hypoplastic marrow. FISH analysis of bone marrow using X- and Y-chromosome specific probes demonstrated that 80% of marrow cells were donor origin. FISH analysis of peripheral blood also showed that 90% of cells were donor-derived. Comparison of Giemsa-stained cell morphology and FISH using the same bone marrow smear slide revealed that genotype of erythroid precursor cells was predominantly male pattern, suggesting hematopoiesis of donor-derived stem cells in recipient bone marrow. Six weeks after transplant the patient developed diarrhea and an erythematous maculopapular skin rash on her back. A diagnosis of acute GVHD was made and treatment with methylprednisolone was instituted. Despite the treatment the patient developed respiratory failure and sepsis and died 46 days after transplantation. Our result showed that FISH analysis using sex chromosome probes is useful to confirm the diagnosis of GVHD following organ transplantation from a donor of the opposite sex. We also found that hematopoietic stem cells in liver graft can migrate to the recipients bone marrow. Further study is required to clarify contribution of donor-derived hematopoiesis to development of GVHD and/or engraftment.

Identification and characterization of a *NUP98-PHF23* fusion gene in acute myeloid leukemia. *J. Reader[1], J. S. Meekins[2], I. Gojo[3,4], Y. Ning[1,2]* 1) Program in Human Genetics; 2) Dept of Pathology; 3) Dept of Hematology-Oncology, University of Maryland School of Medicine, Baltimore, MD; 4) Marlene and Stewart Greenebaum Cancer Center, Baltimore, MD.

NUP98 is a promiscuous fusion partner gene linked to hematological malignancies. We identified a cryptic 11;17 translocation in an acute myeloid leukemia (AML) patient creating a novel in-frame fusion between *NUP98* exon 13 with *PHF23* exon 4. *NUP98* encodes a nucleoporin and has been involved in more than 20 different fusions. *PHF23* is an uncharacterized gene encoding a protein containing a plant homeodomain (PHD) found in chromatin remodeling proteins. The fusion partners of *NUP98* form two distinct groups: homeobox (*HOX*) genes and non-homeobox (non-*HOX*) genes. The non-*HOX* fusion partner genes, which include *NUP98-PHF23*, are diverse in function and are only related by possessing and retaining coiled-coil domain(s). The majority of research has focused on the mechanism of *NUP98-HOX* genes in leukemogenesis; therefore, our interests lie in further characterizing this novel non-*HOX* fusion gene. We hypothesize that *NUP98* fusion genes act as aberrant transcription factors that bind to DNA leading to transcriptional dysregulation. In order to test if *NUP98-PHF23* is able to confer an oncogenic phenotype, we cloned the full length fusion gene into an expression vector and expressed it in fibroblast cell line NIH-3T3 and myeloid cell line K562 for localization, differentiation and chromatin immunoprecipitation (ChIP) analyses. We have shown that *NUP98-PHF23* fusion protein has nuclear localization in NIH-3T3 cells. In K562 cells, the *NUP98-PHF23* fusion protein can partially block TPA-induced differentiation and preliminary ChIP and real time quantitative PCR (Q-PCR) analysis shows increased binding of the fusion protein to the promoter of *HOXA9*. These results indicate that *NUP98*-non-*HOX* fusions may function through *HOX* dependent pathways. Future directions include gene expression studies, identifying potential interacting co-factors and determining whether *NUP98-HOX* and *NUP98*-non-*HOX* have a shared mechanism in leukemogenesis which, in turn, could lead to new potential therapeutic targets.

Meta-analysis of SRD5a2 V89L and A49T polymorphisms and prostate cancer. *J. Li[1,2], R. Coates[2], M. Gwinn[2], M. Khoury[2]* 1) Cancer Division CDC, Atlanta, GA; 2) NOPHG CDC, Atlanta, GA.

The SRD5a2 gene codes for prostatic steroid 5A-reductase type 2, a critical enzyme in the metabolism of androgen. Two SRD5a2 missense substitutions, V89L and A49T, have been studied for association with prostate cancer. A 2003 meta-analysis by Ntais et al found no association with V89L and only a modest association with A49T, which they suggested might be explained by bias. Since 2003, many additional genetic association studies of these variants have been published with conflicting results. We conducted a meta-analysis to reexamine this association. We identified 25 eligible case-control studies published before October 2007, including the 11 analyzed by Ntais et al. We performed a meta-analysis of 22 association studies of V89L (10,088 cases; 10,120 controls) and 16 studies of A49T (5,386 cases and 5,912 controls). To account for variations among studies, we used a random effects model to summarize results. For the V89L polymorphism, we found no increase in risk associated with the L allele compared with the V allele (OR=0.99; 95% CI=0.94-1.05). Results were similar for different racial/ethnic groups. Models contrasting LL with VV found no association with prostate cancer; results were similar for both recessive and dominant models. For the T allele of A49T, we found a small effect that was not statistically significant (OR=1.23; 95% CI=0.94-1.61). The comparison of TT versus AA showed risks ranging from protection to harm (OR=1.21; 95% CI=0.46-3.15) and results were similar for both recessive and dominant models. Our study suggests that the V89L polymorphism is not associated with risk of prostate cancer. Risk related to A49T is uncertain, despite results of 16 studies. Disease selection bias, small sample size, convenience sampling, and genotype misclassification may influence the validity of meta-analysis of these studies. So far, no genome-wide association study of prostate cancer has examined the V89L and A49T polymorphisms directly because neither of them is interrogated by commercial chips in common use. Well designed population-based studies with appropriate genotyping could further elucidate the role of SRD5a2 polymorphisms in prostate cancer.

Genetic Variation in IL6 and LRP5 is Associated with Osteoporosis in the Marshfield Clinic Personalized Medicine Project: Evidence for Interaction with Smoking. *P. Giampietro[1], C. McCarty[1], B. Mukesh[1], F. McKiernan[1], D. Wilson[1], A. Shuldiner[2], J. Liu[2], J. LeVasseur[1], L. Ivacic[1], T. Kitchner[1], N. Ghebranious[1]* 1) Dept Med Genetics, Marshfield Clinic, Marshfield, WI; 2) University of Maryland School of Medicine, Baltimore, MD.

A nested case-control study within the Marshfield Clinic Personalized Medicine Cohort was performed to assess the relative impact of cigarette smoking,statin use,genetic polymorphisms, and one-way interaction of these factors on the development of osteoporosis in post-menopausal women. The study group consisted of 309 postmenopausal Caucasian females with osteoporosis(DXA T-scores -2.5 (spine, femur or radius))and 293 matched controls with normal BMD values. The ethnic composition of the sub population studied was 99.7% Caucasian with predominant German ancestry. Cases differed from controls with respect to BMI (cases: 32.9 +/-6.7 vs. controls: 26.9+/- 5.0 kg/m^2; $p<0.001$), mean age(70.4 + 9.4 vs. 61.6+ 8 years,$p<0.001$)and smoking (70.2% cases never smokedvs.60.1% controls;$p=0.02$), but not statin use. Fourteen SNP alleles corresponding to VDR, ESR1,COL1A1,IL-6,TGF-,ApoE and LRP5 genes,chosen based upon known functional consequences or prior evidence for association in other studies were genotyped using MALDI-TOF. The IL6-C634GC(rs1800796) allele of the promoter region was found to be associated with osteoporosis (odds ratio(OR) for CC+CG = 2.51 (95% CI=1.33, 4.75; $p=0.0047$),independent of statin use or smoking status. This finding replicates the association of rs1800796 with osteoporosis reported by Ota et al.(J Hum Genet 2001;46: 267)in a Japanese population. However,we found the opposite allele associated with osteoporosis suggesting the causative variant may be on a different haplotype in the two populations. On stratification for smoking,an association with LRP5 C135242T (rs545382)and osteoporosis emerged (OR 2.8 in smokers with the CT genotype (95% CI= 1.1, 7.0 $p=0.03$),suggesting a role for an environmental interaction in this association. In conclusion, we provide evidence for a role of genetic variation in IL6 and LRP5 in osteoporosis risk in Caucasian women,the latter manifest only in smokers.

Alterations in gene expression in the MPS VII mouse brain. *R. Rozen[1], D. Smirnov[1], E. Rappaport[1], Z. Zhang[1], E. Cabacungan[1], V. Cheung[1-3], J. H. Wolfe[1,2]* 1) Stokes Research Institute, Childrens Hospital of Philadelphia; 2) University of Pennsylvania, Philadelphia PA; 3) Howard Hughes Medical Institute.

Mucopolysaccharidosis (MPS) VII, Sly disease, is a rare multi-system disease causing mental retardation and death in childhood or early adulthood. MPS VII is one of more than 60 lysosomal storage diseases (LSD). MPS VII is caused by a deficiency in -glucuronidase (GUSB) leading to accumulation of glycosaminoglycans (GAGS) in cells. The murine model of MPS VII shares most of the clinical signs of the human disease. However, little is understood about the cellular and molecular mechanisms involved in mental retardation. Neurological abnormalities in the MPS VII mouse include: 1) behavioral deficits suggesting hippocampus (HC) involvement; and 2) pathological neurodegenerative lesions and astrogliosis in specific regions of the brain including the HC and the cortex (CTX). To determine if specific changes in gene expression and/or pathways contribute to these pathologies, we performed microarray analysis (Affymetrix Mouse 430A 2.0) on HC and CTX samples from 5 and 10 month old normal and MPS VII littermates. We found a limited set of genes (357) showing a 2 fold change ($p<0.01$) in both brain regions which were altered in the MPS VII mice. Changes were seen in lysosomal enzymes and astrocyte activation markers that are consistent with previous pathological findings. Increases in inflammatory markers have been observed in some mouse models of LSD. Changes in neural cell development markers were also seen. A decrease in oligodendrocyte markers suggest alterations in myelination, which are seen in some LSD but have not been reported for MPS. Nine genes were chosen for further analysis: two lysosomal enzymes (GUSB, HEXB), two astrocyte markers (GFAP, LYZS) and five oligodendrocyte markers (ASPA, MBP, MOBP, OLIG2 and PLP1). The changes in direction and magnitude were validated using quantitative RT-PCR (TaqMan). Riboprobes for the nine genes are being constructed to localize changes within the HC and CTX. The data suggest several avenues to peruse in understanding the cellular and molecular basis of mental retardation in a neurogenetic disease.

GenTAC: The National Registry of Genetically Triggered Thoracic Aortic Aneurysms and Cardiovascular Conditions. *C. L. Maslen, and the GenTAC Registry Consortium* Oregon Health & Science U, Johns Hopkins U, U Pennsylvania, U Texas-Houston, Baylor Coll Med, Cornell U, U Michigan, NHLBI, NIAMS, RTI International.

Funded by the National Institutes of Health, GenTAC is a multicenter, longitudinal, observational cohort study of patients at risk for thoracic aortic aneurysms and aortic dissections (TAAD) due to underlying genetic conditions. GenTAC is establishing a registry of clinical data (physical findings, family history, images, and surgical reports) and banked specimens (DNA, plasma, and aortic tissue) from 2800 patients. Clinical data will be updated 2 years after enrollment. The study will compare cross-sectional and longitudinal data regarding phenotypes and risk factors. It will focus on gene-specific phenotypic features, treatment, and outcomes and on identification of imaging methods and plasma biomarkers that can improve the diagnosis and treatment of TAAD. GenTAC began enrollment in November 2007 and should be completed in September 2010. As of May 2008, it comprised 245 patients with a mean age of 34 years (range, 1 to 87 years). Primary diagnoses include Marfan syndrome (MFS), 113 (46%); bicuspid valve (BAV) with aneurysm or a family history of aneurysm, 65 (27%); familial thoracic aortic aneurysm, 34 (14%); idiopathic TAAD in patients less than 40 years, 25 (10%); Loeys-Dietz syndrome, 6 (2%); Turner syndrome, 7 (3%); and Ehlers-Danlos syndrome, 7 (3%). Some patients fit into more than one diagnostic category. The current registry comprises 224 Caucasian (91%), 8 Hispanic (3%), 6 Asian (2%), 6 black (2%) patients, and one of Pacific descent. The mean age at diagnosis was 21 years (range, 0 to 72 years). A total of 69 dissections occurred in 29 patients (9 with MFS, 10 with familial TAAD, 6 with BAV, and 4 with idiopathic TAAD), and 129 have a history of surgical intervention. Researchers have collected blood samples from 202 patients and saliva specimens from 59 patients. GenTAC will be a rich source of clinical and genetic data that will increase our understanding of the genetic basis and clinical management of TAADs. Investigators interested in using GenTAC for ancillary studies should contact the registry at gentac-registry@rti.org or http://gentac.rti.org.

A Heterozygote-Homozygote Test of Hardy-Weinberg Equilibrium. *J. S. Sinsheimer[1,2], J. J. Zhou[1], J. C. Papp[2], K. Lange[1,2]* 1) Dept Biomathematics, Univ California, Los Angeles, Los Angeles, CA; 2) Dept Human Genetics, Univ California, Los Angeles, Los Angeles, CA.

Linkage and association methods typically rely on the principle of Hardy-Weinberg equilibrium (HWE). Although a number of HWE tests exist, most cannot be applied when subjects are related or when the markers are non-codominant. We develop the Heterozygote-Homozygote (HH) test, which can be used with both pedigrees and individuals. In the HH test, the homozygote-heterozygote ratio is estimated by maximizing the likelihood using an efficient quasi-Newton algorithm. The analysis of over 1250 highly polymorphic, multi-allelic markers using extended CEPH pedigrees takes on the order of an hour on a PC with ~130 genotyped individuals where some founder genotypes are missing. Non-codominant markers present no computational difficulties; therefore tightly linked snps can be used in combination without first estimating individuals haplotypes. Using both simulations and actual data examples, we find that the HH test has the appropriate type I error rate and excellent power.

Imatinib Response in CML Patients with Deletions on the Derivative Chromosome 9. *A. L. Berger-Zaslav[1], S. Spitzer[2], T. Mercado[1,2], S. Richard[3], T. Pardee[3], E. Knorr[1], D. Tully[1], K. Zamkoff[3]* 1) Cytogenetics, UHMC, Stony Brook, NY; 2) Molecular Genetics, UHMC, Stony Brook, NY; 3) Blood and Marrow Stem Cell Transplantation Program, UHMC, Stony Brook, NY.

Ph results from a reciprocal (R) translocation (T) of chromosomes 9 and 22. The BCR/ABL gene is formed on the der(22) and the ABL/BCR gene on the der(9). FISH identified unexpected deletions (D) of the T product in 10-15% of patients (Pts) with CML. Studies have shown atypical (AT) abnormal (AB) findings were associated with a more rapid progression (P) to blast crisis (BC) and a shorter overall survival. We previously reported two cases of CML with a D of the ABL/BCR on the der(9). We present follow-up data on these Pts and a third. Initially all Pts were placed on hydroxyurea (H) and allopurinol followed by imatinib therapy (IT). Pts were monitored every 3 m for a y. Pts were evaluated using standard cytogenetic techniques (20 metaphases (Ms) when possible); FISH (200 nuclei (N) each; BCR/ABL DCDF Abbott Mol.), and by quantitative PCR (Roche Biochem.). Initial results were Pt 1: 25 y male in chronic phase (CP) with the T in all Ms, the AT in 194/200 N, and a BCR/ABL/G6PDH ratio of 1.8. Pt 2: 44 y male in CP with the T in all Ms, and a del(6)(q13) in 6/20 Ms, the AT in 159/200 N and a BCR/ABL/G6PDH ratio of 0.2. Pt 3: 57 y female in BC, chromosome analysis was not possible, the AT was present in 157/200 N and a BCR/ABL/G6PDH ratio of 1.8. After induction Pt 1 had a partial cytogenetic response (CR) but no decrease in the BCR/ABL/G6PDH ratio. Pts 2 and 3 showed a significant CR, and had a molecular response (MR) by a 3 log decrease. After 9 m Pt 1 had a partial CR and no MR. Pt 2 achieved remission (RE). Pt 3 never achieved RE. Ds on the der(9) may be a poor prognostic indicator (PI). Early studies were based on H or interferon. Recent studies have demonstrated that this is also true for D Pts on IT. All Pts reported here have AT DS on the der(9). Two of 3 Pts (1 and 3) had a poor response to IT. Even though our Pt sample is small the data corroborates that Ds on the del(9) maybe a poor PI. More studies of Pts with Ds will be useful in determining the significance of these findings.

Frequency of XRCC1 Arg194Trp polymorphism in lung cancer Mexican patients. *MP. Gallegos[1], JM. García[1], G. Morgan[2], AM. Puebla[1], G. Zuñiga[1]* 1) Dept Med Molec, Guadalajara, CIBO, IMSS, Jalisco, Mexico; 2) Centro de Radio Neurocirugía, Hospital de Especialidades, CMNO, IMSS.

Lung cancer is a cause of multiple investigations worldwide due to the high incidence in developed countries and developing. In Mexico it presents as a major health problem, just in the state of Jalisco in 2005 ranked fifth death by cancer, with a frequency of 12.66%. Based on the foregoing different studies in literature have focused on the role they have systems of DNA repair (BER) in cancer and have described the association between Arg194Trp polymorphism in the gene XRCC1 with lung cancer in different populations world. However, there are contradictions in this regard. In Mexico there are no studies of the association between Arg194Trp polymorphism in the gene XRCC1 with lung cancer, which is why in through a descriptive study analyzed 100 samples of DNA Genomic patients with lung from the Hospital de Especialidades, CMNO, IMSS. Guadalajara Jalisco. Mexico. As well as 100 controls samples from Jalisco general population. By PCR a fragment of 491 bp was amplified and identified by Msp I restriction enzyme on electrophoresis gels polyacrylamide to 6% (29:1) after staining with silver nitrate. The frequency of genotypes: Trp / Trp was 4% (5 / 100) and 1% (1 / 100); the Arg / Trp 25% (25/100) and 21% (21/100) and Arg/Arg 70% (70/100) and 78% (78/100) in patients and controls respectively without showing association between the Arg194Trp polymorphisms in the gene XRCC1 with lung cancer [OR 5.21 (CI95% 0.56-248.98)] in the tested sample, so it is concluded that this polymorphism is not associated directly with lung cancer of the tested sample of the Mexican population.ñ.

Arg194Trp polymorphism XRCC1 of gene is not associated with breast cancer from Western of Mexico. *MJ. Renteria[1], M. Morgan[3], AM. Puebla[1], D. Ontiveros[2]* 1) Medicina Molecular, CIBO, IMSS, Guadalajara, Jalisco, Mexico; 2) Hospital de Gineco-Obstetricia, CMNO,IMSS, Jalisco; 3) Centro de Radio-Diagnostico, Hospital de Especialidades, CMNO, Jalisco.

Breast cancer as health problem worldwide for this reason multiple investigations to achieve understand its pathogenesis with the aim of halting their advance. In Mexico is a major cause of death in women between 35 to 50 years, only in the state of Jalisco in 2006 year the 67% (86/128) of deaths reports were for breast cancer. Based on the foregoing different studies in literature have focused on the role they have systems of DNA repair (BER) in cancer and have described the association between polymorphism Arg194Trp in XRCC1 gene with breast cancer in different populations in the world. However, there are contradictions in this regard. In Mexico there are not studies of the association between Arg194Trp polymorphism in the gene XRCC1 with breast cancer, which is why in through a descriptive study analyzed 156 samples of DNA genomic of women with breast cancer from the Hospital de Especialidades del CMNO, IMSS Guadalajara, Jalisco; Mexico, and samples of 100 women controls the general population of Jalisco. By PCR amplification a fragment of 491 bp and the identification of polymorphism were performed by restriction enzyme MspI means electrophoresis gels polyacrylamide to 6% (29:1) after staining with silver nitrate. The frequency of genotypes: Trp / Trp was 4% (6 / 156) and 1% (1 / 100); the Arg / Trp 17% (26/156) and 21% (21/100) and Arg / Arg 79% (124/156) and 78% (78/100) in patients and controls respectively without showing association between polymorphisms of Arg194Trp of XRCC1 gene in breast cancer patients [OR 3.96 (CI95% 0.47-183.93)]. In the sample analyzed, so it is concluded that this polymorphism is not associated directly with the breast cancer of the tested sample of the Mexican population.

Postnatal diagnosis of 46,XY/45,X mosaicism in dichorionic/diamniotic monozygous twins with discordant gender and somatic phenotypes. *A. M. Svensson[1,2], S. T. South[2,3], A. Rope[3]* 1) Dept. of Pathology, Univ. of Utah & ARUP Laboratories, Salt Lake City, UT; 2) ARUP Laboratories, Salt Lake City, UT; 3) Division of Medical Genetics, Department of Pediatrics, University of Utah Health Sciences Center, Salt Lake City, UT.

We describe a pair of dichorionic, diamniotic, monozygous twins with different ratios of 46,XY/45,X mosaicism. At birth, the female twin displayed mildly ambiguous genitalia and coarctation of the aorta. She died in the newborn period from necrotizing enterocolitis. Her karyotype, performed on stimulated peripheral blood, demonstrated 45,X/46,XY mosaicism, with 45,X in 10 cells and 46,XY in 10 cells. Her male twin's karyotype was evaluated simultaneously and also showed 45,X/46,XY mosaicism with 45,X in 3 cells and 46,XY in 47 cells. Zygosity analysis on DNA from peripheral blood, utilizing a panel of 15 short tandem repeat markers and amelogenin, demonstrated monozygosity. No other cell types were available for analysis in either twin. At the age of 3½ months the male twin showed normal development, with phenotypically normal external male genitalia. Apart from a small umbilical hernia, no other phenotypical abnormalities were seen. The occurrence of dichorionic, diamniotic, heterokaryotypic, monozygotic twinning with discordant sexual and somatic phenotypes due to different ratios of mosaicism is extremely rare. Dichorionic, diamniotic, monozygotic twins are assumed to have separated very early in embryogenesis. Possible mechanisms behind the generation of mosaicism in monozygotic twins include mitotic nondisjunction leading to loss of the Y chromosome. Skewed spatial allocation of the abnormal cells may then cause the two cell populations to recognize each other as different and split into two distinct cell masses. Any twin of a sibling demonstrating 45,X/46,XY mosaicism should be evaluated for this rare, yet possible, phenomenon to ensure appropriate screening and medical management, as well as to provide accurate recurrence risk counseling.

IL-1ra VNTR polymorphism frequency in breast cancer patients and controls from western of Mexico. *AM. Puebla[1], H. González[1], G. Morgan[2], D. Ontiveros[3], MP. Gallegos[1]* 1) Medicina Molecular, CIBO, IMSS, Guadalajara, Jalisco, Mexico; 2) Centro de Radio-Neurodiagnostico, Hospital de Especialidades, CMNO, IMSS, Jalisco; 3) Hospital de Gineco-Obstetricia, CMNO, IMSS, Jalisco.

Breast cancer is a health problem globally, is the most frequent cause of death among women 35 to 50 years in Mexico, regarded as the leading cause of death among females. These forced our society to carry out studies to understand the pathophysiology of the disease to stop their advance and find the cure more effective. Actually have been studies on the participation of interleukin as proinflammatory molecule in the pathogenesis of cancer. In the present study we investigate the frequency VNTR polymorphism in the gene IL-1ra in120 patients with breast cancer from Hospital de Gineco-Obstetricia, CMNO, Guadalajara, Jalisco, Mexico, and 118 healthy subjects from general population of Guadalajara. Through the PCR were amplified the VNTR alleles 1 (442bp, 4 repetitions), 2 (270bp, 2 repetitions) and 4 (365bp, 3 repetitions) of gene IL-1ra. Posteriori were identified through in polyacrylamide (6%) gels electrophoresis after staining with silver nitrate. The frequency for the 1 allele was 0.38 and 0.56 and allele 2 of 0.52 and 0.44 respectively in patients and controls. The alleles 3 and 4 were observed only in breast cancer showing a frequency of 0.05 for both alleles. Showing significant differences $p < 0.05$. The frequency of 1 allele of the gene VNTR IL-1ra in the control group was higher in comparison to breast cancer patients showing as a protective allele in the tested sample of Mexican population [OR of 0.45 (CI95% 0.25 to 0.82)].

Integration and systems analysis of the genetics of common human multigenic disease. *S. De[1], Y. Zhang[1], J. R. Garner[1], S. A. Wang[2], K. G. Becker[1]* 1) Gene Expression and Genomics Unit, RRB, National Institute on Aging, National Institutes of HealthBaltimore, MD 21224; 2) Division of Computational Bioscience, Center for Information Technology, National Institutes of Health, Bethesda, MD 20892.

Complex multigenic diseases such as cardiovascular disease, autoimmune disorders, neurological disorders and metabolic diseases make up a majority of mortality and morbidity in developed countries. In this study, positive disease associations from the Genetic Association Database (GAD) (Becker et al. 2004) as well as mouse genetic and phenotypic data from the mouse MGI database were used. Disease associated genes and mouse gene-phenotypes were were compared using a unique method similar to phyologenetic classification. First the distance between the diseases were calculated by pairwise comparison of the genes associated with specific diseases. The disease/phenotype relationships were calculated from the distance matrix using Fitch based on the Fitch and Margoliash method of constructing phylogenetic trees. The Neighbor-Joining method of Saitou and Nei (1987) was also used to visualize larger sets. Although Fitch performed more consistently in randomized inputs, Neighbor gave very similar results. A more traditional method of Hierarchical Clustering was also used to determine distance relationships between human disease associated gene sets as well as mouse phenotype gene sets. This approach, based on gene sharing, identified major groupings of disease, placing related disorders in appropriate general disease/phenotype categories, as well as positioning highly related disorders closer in space. This was true for 480 common human diseases, as well as for genes related to 1,056 mouse phenotypes. In addition, we have used these human and mouse disease/phenotype gene sets to rapidly and systematically interrogate public disease based microarray datasets showing disease specificity. This analysis suggests higher order structure and disease relevance in genetic associations mined from published literature. This approach may be developed to make predictions regarding aggregate multigenic risk of developing related common complex disorders. Supported by the IRP of the NIA,and CIT, NIH.

Efficacy of clinical diagnosis of chromosome disorders in cases with dysmorphic features and the cytogenetic findings: A study of 438 cases with dysmorphic features. *F. M. Badr[1,2], K. AlSaidi[2], K. AlMuteri[2], M. M. AlSamman[2], S. S. Binhassan[2]* 1) Faculty of Medicine, King Fahad Medical City, Riyadh, KSA; 2) Cytogenetics and Molecular Cytogenetics Laboratory, King Fahad Medical City, Riyadh, KSA.

Objectives : To report the incidence and distribution of chromosome disorders confirmed by different cytogenetic techniques among cases with dysmorphic features clinically diagnosed as genetic disorders. Subjects and Methods : A total of 438 newborn babies and infants up to 4 years old were referred by clinicians for cytogenetic analysis. Indications for referral were multiple congenital anomalies, and a wide spectrum of dysmorphic features. All cases were karyotyped by conventional G-banding technique and fluorescence insitu hybridization (FISH) was done on interphase cells or on metaphase chromosomes. Results : 137 cases were diagnosed clinically as specific chromosome disorders. All cases referred as Edward, Patu's syndromes (trisomy 13 & 18) and Klinefelter were positively confirmed by cytogenetic testing. Only 57 cases out of 100 clinically diagnosed as Down's syndrome (trisomy 21) were confirmed cytogenetically. Concordance between clinical diagnosis of cases as Turner syndrome with karyotype analysis was very low (5%). Non specified dysmorphic feature cases revealed an incidence of 10.7%; with structural chromosomal aberrations. Of the latter, deletions were the most frequent (37%), translocation (7%), isodicentric ((7%), duplication (21%), marker chromosome (7%), and complex rearrangements (21%). Conclusion: The highest concordance ratio between clinically diagnosed cases and positive cytogenetic findings included chromosome disorders with well recognizable symptoms as the case with trisomies 13, 18 & 21. The low concordance ratio between referral cases and chromosomal abnormalities could be due to the inclusion of cases which mimic phenotypic description of clinically recognizable chromosome syndromes. Other factors which lead to suspicion of chromosome aberration are increased maternal age, consanguinity and familial history.

An adult Acute Leukemia Diagnosed as either AML M_4 or M_5 by Morphologies; and as Non-M_3 AML by Flow Cytometry; the Result was Revised as AML M_{3v} Based on Final Cytogenetic Findings. *H. O. Shah[1, 2], J. Z. Tao[1], D. Mockler[1], S. Naik[1], J. H. Lin[1, 2, 3]* 1) Dept Pathology, Cytogenetics, Nassau Univ Med Ctr, East Meadow, NY; 2) Health Sciences Center, Stony Brook, SUNY; 3) New York College of Osteopathic Medicine, NY.

A 43-year old woman with a history of HIV was admitted to our hospital with a 2-day fever and dehydration. The peripheral smear showed a marked leukopenia (0.6 K/mm^3) and majority of leukocytes were immature showing monocytoid nuclear features characterized by paler, lacy chromatin, occasional reniform and bilobed nuclei and nucleoli. The cytoplasm showed sparse fine granules with no apparent Auer rods. The flow cytometry of immature cells from peripheral blood were positive for CD34, CD33, CD117, CD13 markers, and negative for CD4, CD38, HLA-DR, and B and T lymphoid markers. The marrow flow cytometry showed similar results. A preliminary diagnosis non-AML M_3, of acute myelogenous leukemia of monocytoid features (either M_4 or M_5) was made. Three days following the admission, cytogenetic result of classic 46, XX,t(15;17) (q22;q21) was obtained. The patient was then treated with the protocol for AML M_3. AML M_3 comprises about 5 - 10 % of all AML with a median age of 35 - 40 years old. Morphologically, M3 variant of AML (AML M_{3v}) show fine, sparse cytoplasmic granules, even though specific esterase can elucidate abundant reaction in cytoplasm. Initial leukopenia is most commonly seen in classic M_3, however there is higher frequency of clinical disseminated intravascular coagulation with bleeding in AML M_{3v} . On the contrary to this case, the patient showed initial severe leukopenia with non-classic blasts and no bleeding episodes.

Fetal phenotype of four cases of campomelic dysplasia harbouring novel mutations of SOX9 gene. *F. Lalatta[1], B. Gentilin[1], F. Forzano[2], F. Faravelli[2], M. Baffico[2], M. Lituania[3], P. Ficarazzi[4], T. Rizzuti[5], P. Bianchi[6], E. Grosso[7], M. F. Bedeschi[1]* 1) Medical Genetics Unit, Fondazione IRCCS Ospedale Maggiore Policlinico Mangiagalli e Regina Elena,Milano, Italy; 2) Medical Genetics Unit; E.O. Ospedali Galliera, via Volta 6 Genova Italy; 3) Fetal Medicine Unit; E.O. Ospedali Galliera, via Volta 6 Genova Italy; 4) Obstetrics and Gynecology Unit; Fondazione IRCCS Ospedale Maggiore Policlinico Mangiagalli e Regina Elena,Milano, Italy; 5) Patology Unit;Fondazione IRCCS Ospedale Maggiore Policlinico Mangiagalli e Regina Elena,Milano, Italy; 6) Pediatric Unit; Ospedali Riuniti di Bergamo, Largo Barozzi 1, Bergamo Italy; 7) SCDU Medical Genetics Ospedale S. Giovanni Battista (Molinette) Torino Italy.

Campomelic dysplasia (CD) is a rare congenital skeletal disorder characterized by bowing of the long bones and a variable association of other skeletal and extraskeletal defects, with or without XY sex reversal. CD is caused by mutations in the SRY-box 9 gene (SOX9), a dosage-sensitive gene expressed in chondrocytes and other tissues and located at 17q24. The genotype and phenotype correlation of campomelic dysplasia is still unclear. In the prenatal period the most characteristic sign of campomelic dysplasia is the shortening and marked anterior bowing of long bones, particularly of femur and tibia. Narrow chest, scoliosis, talipes equinovarus, and flat facial profile are other sonographic features commonly present. Increased nuchal translucency, polyhydramnios, and anomalies of the central nervous, cardiac, and renal systems have also been described. We report four cases of campomelic dysplasia detected before the 22nd week of gestation and suspected in the first or second trimester of pregnancy by prenatal ultrasound. The pregnancies were all terminated and the diagnosis of campomelic dysplasia has been confirmed on clinical and radiographic examination of the fetuses. In all cases molecular analysis detected three novel mutations in the SOX9 gene which occurred de novo. Subsequent investigation demonstrated the pathogenetic nature of these mutations.

Inference of haplotypic phase and missing genotypes in polyploid populations and copy number variation regions. *S. Y. Su, D. J. Balding, C. J. M. Coin* Department of Epdimiology and Publuc Health, Imperial College Loncon, London, United Kingdom.

Haplotype-based approaches are powerful tools for association studies, linkage disequilibrium(LD) studies, analysis of copy number variation (CNV) and ancestral inference. However, carrying out a haplotype-based analysis on the basis of genotype data is difficult in polyploid organisms due to lack of an appropriate phasing program. Polyploidy is common in plants (e.g. potato, wheat) as well as in some animals (e.g. goldfish, salmon). Polyploidy also occurs in humans, one such as example is trisomy 21--a common genetic disorder causing Down syndrome. Moreover, heart and liver cells are known to have variable ploidy, as do cancer cells. Copy number variation also results in `local changes in ploidy along the genome. Here, we present a method and software for inference of haplotype phase and missing genotypes from genotype data in a polyploid population. Each sample in the population can have a different ploidy. Our method employs a hidden Markov model (HMM) and a sampling algorithm to infer haplotype structure jointly in multiple samples. Our method also accurately calculates the uncertainty in this estimate. To evaluate the performance of our method, we pair real haploid genotype data to create artificial diploid, triploid, and tetraploid genotype data of known haplotypic phase. The results of the simulation study demonstrate that our method for phasing polyploid genotypes performs well in terms of switch error rate and imputation error rate. Comparing with existing methods, our method is more accurate than fastPHASE for diploids, while there is no existing program available for haplotype phase inference in triploids and tetraploids.

Prenatal manifestation and management of mother and child affected by Spondyloperipheral dysplasia with a C-propeptide mutation in COL2A1. *M. F. Bedeschi[1], V. Bianchi[1], L. Colombo[2], F. Natacci[1], S. Giglio[3], E. Andreucci[3], L. Trespidi[4], B. Acaia[4], A. Superti-Furga[5], F. Lalatta[1]* 1) Clinical Genetics Unit, Fondazione IRCCS Ospedale Maggiore Policlinico Mangiagalli e Regina Elena, Milan, Italy; 2) Neonatal Intensive Care Unit, Fondazione IRCCS Ospedale Maggiore Policlinico Mangiagalli e Regina Elena, Milan, Italy; 3) Department of Clinical Pathophysiology and Medical Genetics Unit, AOU Meyer, Univ. of Florence, Florence,Italy; 4) Obstetrics and Gynecology Clinic I, Fondazione IRCCS Ospedale Maggiore Policlinico Mangiagalli e Regina Elena, Milan, Italy; 5) Dept. of Pediatrics, Univ. of Freiburg, Germany.

It is not unusual for patients with rare conditions to remain undiagnosed until adulthood. In such cases, a pregnancy may result in unexpected problems and special needs. A 28-year-old primigravida was referred at 17 weeks gestation for counselling for possible Dyggve-Melchior-Clausen syndrome, a recessive disorder. She had a skeletal dysplasia with a flat face, short trunk, scoliosis and lumbar hyperlordosis, platyspondyly, and short toes II to V, suggesting the diagnosis of a collagen 2 disorder and specifically of spondyloperipheral dysplasia (MIM 156550). The proband was counselled about the probability of dominant rather than recessive inheritance and offered prenatal diagnosis by sonography. Serial US examinations at 17 to 20 weeks showed fetal macrocephaly, narrow thorax, and shortening and bowing of long bones. The parents opted for continuation of the pregnancy. The baby was delivered at week 33 for maternal reasons, had severe respiratory distress for four weeks but eventually recovered. Mutation analysis revealed a heterozygous nonsense mutation in the C-propeptide coding region of COL2A1 (c.4339 A>T, K1447X) confirming the diagnosis of SPD as well as the geno-pheno correlations between C-terminal COL2A1 mutations and the SPD-Torrance spectrum (Zankl et al, AJMG 133A:61-67, 2005). This case illustrates the importance of a correct diagnosis in adulthood, when individuals with rare conditions have a need to know about recurrence risk, pregnancy-associated risks, as well as potential complications in the newborn.

DETERMINATION OF DNA INDUCED DAMAGE WITH PARACETAMOL IN HUMAN AMNIOCYTES WITH NORMAL AND ABNORMAL KARYOTYPE BY UNICELLULAR ELECTROPHORESIS. *R. Báez-Reyes[1,3], JC. Báez-Reyes[2], G. Razo-Aguilera[1,3]* 1) Deparment of Genetics, National Institute of Perinatology; 2) Deparment of Histophatology, UNAM; 3) Deparment of Morphology, Biologhical Sience School, IPN. Mexico,City.

INTRODUCTION: The unicellular electrophoresis (UE) or comet assay is a very sensitive method to detect the direct damage to the DNA.. The migration of the DNA or tail of the comet represent the damage taken place by the toxic agent. OBJECTIVE: To determine the damage to the basal and induced DNA with paracetamol by means of the UE in amniocytes with normal and abnormal karyotype. MATERIAL AND METHODS: We took a sample of amniotic liquid from 30 women for cytogenetic study. We realized G bands for cytogenetic analysis. Later on, subcultures were made to be treated with 13g/ml of paracetamol during 0, 15, 30 and 45 min. Before and after each time of exhibition the cells were tripsinizing and absorbed in agarose gels to be processed by mean of UE. It was calculated the average of migration of the DNA and the standard error, the results were compared with the Student t test. RESULTS: 15 fetuses had normal karyotype (8 male and 7 female) and 15 had abnormal karyotype. With regard to the basal damage we obteined a migration of the DNA in non cultivated amniocytes of: 2.19 + 0.98 m for the normal fetuses and of 1.44 + 1.77 m for the abnormal ones. In the cultivated amniocytes of: 2.55 + 3.24 for the fetuses with normal karyotype and of 3.44 + 1.61 m for fetuses with abnormal karyotype. In relation to the damage induced by the paracetamol a migration of DNA it was observed: 3.25 + 0.23 m (control), 14.49 + 5.19 m (15 min), 19.62 + 11.4 m (30 min) and 4.11 + 1.01 m (45 min) for the cases with normal karyotype and of: 2.17 + 0.97 m (control), 24.71 + 13.56 m (15 min), 20.48 + m (30 min) and 6.31 + 10.6 m (45 min) for the cases with abnormal karyotype. CONCLUSIONS: The unicellular electrophoresis is an useful methodology to value the damage to the basal DNA and induced by paracetamol in amniocytes of fetuses with normal and abnormal karyotype.

Two Adult Siblings with Arginine:Glycine Amidinotransferase Deficiency: further characterization of clinical and biochemical phenotype. *K. Wierenga[1], M. Dowling[1], A. Verma[1], S. Stabler[2], S. H. Zeisel[3], D. Dimmock[4], L.-J. Wong[4], C. Wagner[5], S. H. Mudd[6]* 1) University of Miami, Miami, FL; 2) University of Colorado, Denver, CO; 3) UNC, Chapel Hill, NC; 4) Baylor College of Medicine, Houston, TX; 5) Vanderbilt Univ., Nashville, TN; 6) Lab Mol Biol, NIMH, Bethesda, MD.

Arginine:glycine amidinotransferase (AGAT) catalyzes the first enzymatic step in the biosynthesis of creatine by converting arginine and glycine to ornithine and guanidinoacetate (GAA). AGAT deficiency results in cellular creatine deficiency, as initially described in 4 members of an Italian family. We now report a second family. A female and male sib pair (now aged 26 and 23 years), children of first-cousin parents, had developmental delay with severe speech delay in childhood. At age 16, the sisters adaptive behavior score was 69; at age 13, the brothers IQ was 40. At age 25, the sister developed progressive proximal muscle weakness. Serum creatine kinase and muscle biopsy were unremarkable. Empiric creatine supplementation (10 g/d) resulted in normalization of muscle strength; communication skills and cognition however showed little improvement. GAA was below the levels for detection in urine and plasma, consistent with a diagnosis of AGAT deficiency. Evidence that these sibs are homozygous for a deleterious AGAT gene mutation will be reported elsewhere at this meeting. After 7 months on creatine therapy, NMR spectroscopy showed brain creatine peaks of 40% of normal in both patients. Concurrent IQs were 53 and 50, respectively. Although AdoMet utilization for methylation of GAA must be impaired in these patients, fasting plasma AdoMet was not elevated, nor was methionine. AdoHcy, cystathionine, total cysteine, serine, arginine, ornithine, and sarcosine were normal. Total homocysteine was low-normal. Free choline was high; betaine was low. Phosphatidylcholine and sphingomyelin were low-normal. In conclusion, AGAT deficiency should be considered in patients with mental retardation, especially those who also develop proximal myopathy. Creatine supplementation can reverse the myopathic deficit in AGAT deficiency. Whether significant cognitive improvement occurs following long-term creatine therapy remains unknown.

A patient with Iris Coloboma with Ptosis, Hypertelorism, and Mental Retardation Syndrome showing unusual Brain Malformation. *S. Sakazume[1], Y. Narumi[1], S. Sato[3], K. Maruyama[3], T. Takagi[3], M. Watanabe[2], A. Nishi[4], T. Shiihara[2]* 1) Dept Genetics, Gunma Childrens Med Ctr, Shibukawa, Gunma, Japan; 2) Dept neurology, Gunma Childrens Med Ctr, Shibukawa, Gunma, Japan; 3) Center of peinatal medicine, Gunma Childrens Med Ctr, Shibukawa, Gunma, Japan; 4) Dept surgery, Gunma Childrens Med Ctr, Shibukawa, Gunma, Japan.

We present a patient with Iris Coloboma with Ptosis, Hypertelorism, and Mental Retardation Syndrome (ICPHMR) having band heterotopia in cranial MR. The case reports of this syndrome is limited and causative genetic defect has not been known yet. Pathological and imaging study of brain may leard to confirm syndrome criteria in a future. Baraitser and Winter (1988) described one familial, brother and sister, and sporadic cases diagnosed as Iris Coloboma with Ptosis, Hypertelorism, and Mental Retardation Syndrome (ICPHMR). In three literatures, chromosome anomalies were reported associated with ICPHMR. Pallotta (1991) reported a boy with same phenotype having pericentric inversion of chromosome 2: inv(2)(p12q14), Ramer et al. (1995) assembled two of the individuals having identical pericentric inversions involving 2p12-q14. Now we know that patient reported by Ayme et al. (1979) having pericentric inversion in chromosome 2. One of the candidate genes of ICPHMR is PAX8 which may work as a key gene for forebrain construction. Surely some patients with ICPHMR reported to have brain anomaly, especially lissencephaly. Data accumulations related to brain anomalies are important to ICPHMR gene cloning. This patient is a boy borne with uneventful delivery following 41 weeks of gestation. Multiple anomalies were noted with characteristic face including iris coloboma, hydronephrosis, and lissencephaly. Using University of Ryu-Kyu Data Base for Malformation Syndrome (URDBMS), ICPHMR is the most likely diagnosis considering his facial appearance. Cranial MR at 5-day-old revealed diffuse pachygyria with sub cortical band heterotopia, and periventricular heterotopia was also noted. The molecular studies including genome array should be planned for further diagnosis.

Alternative methods to detect gene-gene interactions. *L. De Lobel[1], H. De Meyer[1], G. Baele[1], M. Kogevinas[3], k. Van Steen[2]* 1) Department of Applied Mathematics and Computer Science, Ugent, Belgium; 2) Department of electroengineering, Université de Liège,Belgium; 3) Respiratory and Environmental Health Research Unit, Municipal Institute of Medical Research (IMIM), Barcelona, Spain.

The search for susceptibility loci in gene-gene interactions imposes a methodo- logical and computational challenge on statisticians due to the large dimensionality of the modeling of epistasis. In a time where genome-wide scans are common, new powerful methods are required to handle the huge amount of feasible gene-gene in- teractions. A solution is to reduce data by preliminary screening of the markers to select the best candidates for further analysis. When the screening technique and statistical analysis are independent, the same data can be used to conduct both analyses. A method to detect gene-gene interactions developed for rather small data is the Multifactor Dimensionality Reduction method (MDR). MDR is a nonpara- metric, model-free data reduction technique that has become a standard in epistatic research. In this study, we examined the power of MDR in larger data and compared it to other approaches. To improve the performance of MDR in this setting, we used Random Forests (RF) as screening method before executing MDR. We found that the power of MDR increased when noise is rst removed by creating a collection of candidate markers with RF. Apart from a simulation study, the approach is also applied to asthma data from the ECRHS study.

Qualitative analysis reveals differential expression of splice variants of TGIF1 gene in oral squamous cell carcinoma. *T. Liborio[1], F. C. A. Xavier[2], L. F. Matizonkas-Antonio[2], M. G. Silva-Valenzuela[3], D. M. Carraro[4], L. P. Kowalski[4], F. A. Soares[4], J. Câmara[1], F. D. Nunes[2]* 1) Pathology Department, Federal University of Amazonas, Manaus, Brazil; 2) Molecular Pathology Laboratory, School of Dentistry, University of São Paulo, Brazil; 3) Biochemist Department, School of Chemistry, University of São Paulo, Brazil; 4) Hospital Ac Camargo, São Paulo, Brazil.

TGIF1 homeobox gene is subject to the alternative splicing process, which represents an important molecular mechanism responsible for proteome complexity. Different splice variants (SV) may be associated with distinctive behaviors in cancer. TGIF1 transcripts were previously detected in oral squamous cell carcinoma (OSCC), although possible divergences of SVs expression are currently unknown. Expression of different SVs of TGIF1 in OSCC was compared to the adjacent non-tumoral margin (NT) and was associated with clinical and histological parameters. Expression of TGIF1 generic protein was also analyzed. Forty eight samples of OSCC and 12 of NT were used. Total RNA was extracted using TRizol solution. Transcripts of TGIF1 were previously amplified by RT-PCR using a generic primer and then submitted to specific primers for SVs 1, 2, 5, 7 and 8. The protein expression was analyzed by immunohistochemistry in 46 paraffin-embedded tissues. All SVs have the same expression frequency in both OSCC and NT samples. However, SVs are differently expressed in the OSCC patients due to association between SVs 1/4 (p-value: 0,001), 1/8 (p-value: 0,02), 2/4 (p-value: 0,017), 4/5 (p-value: 0,032), and 4/8 (p-value: 0,023). It was not observed association between SVs expression with clinical and histological parameters. Kaplan Meier univariate analysis revealed correlation of SVs 1 (log-rank: 0,0769) and 8 (log-rank: 0,0958) with patients survival, both correlated with a lower risk of death. The simultaneous expression of TGIF1 protein not only in the nucleus but also in the cytoplasm of the cell was correlated with poorly differentiated OSCC (p-value: 0,05). SVs of TGIF1 are differentially expressed in OSCC, possibly suggesting different roles for them in oral carcinogenesis.

Inverted duplication with terminal deletions: toward the identification of a preponderant mechanism. *S. Leclercq[1], MC. De Blois[2], E. Pipiras[3], G. Quenum[2], C. Dupont[3], F. Baverel[1], A. Lebbar[1], JM. Dupont[1]* 1) AP HP, Unité de Cytogénétique, Hôpital Cochin-Saint Vincent de Paul, Paris; 2) AP HP, Service de Cytogénétique, Hôpital Necker, Paris; 3) AP HP, Service de Cytogénétique, Hôpital Jean-Verdier, Bondy.

Inverted dupliation with terminal deletion (inv dup del) are complex chromosomal rearrangements. Two mechanisms are proposed to explain their origin. The best known case involves the short arm of chromosome 8. Inv dup del 8p arise through non allelic homologous recombination (NAHR) between two misaligned olfactory receptor (OR) gene cluster. From the distal to the proximal 8p region, the inv dup del 8p comprises three segments: one deleted, one present as a single copy and one inverted duplicated. The second proposed mechanism involves a U type exchange, leading to end-to-end fusion. The initial event would be a DNA double strand break in a subtelomeric region leading to chromosome instability. In this case, no single copy region would be observed between the deleted and duplicated regions. Inv dup del are now reported for an increasing number of chromosome ends. Usually they are isolated cases with a restricted molecular cytogenetic study so the 8p mechanism could not be extended to other chromosome ends. We report the results of a retrospective study of 5 inv dup del cases involving 4q, 11p, 18p, 20p and Xq ends aiming to establish a main mechanism of formation. We used oligo-array (NimbleGen HG18-WG Tiling 385K) tiling the full genome at a median probe spacing of 6.000 bp in order to detect any single copy region. In three out of five cases, no single region is detected, the NAHR mechanism could be excluded. It seems to be more appropriate to suggest a U type exchange mechanism. The oligo-array data enable us to localize the initial theoretical break. In none of our studied cases, this locus is associated with a hotspot breakpoint or a fragile site. Thus, an accidental break probably generated these rearrangements. To date, it seems difficult to propose a unique mechanism. Further exploration among a more important cohort will be necessary to precise frequency of each mechanism.

Identification of a Patient with Partial Hexasomy for the Prader-Willi Angelman Syndrome Critical Region Due to a Tricentric Supernumerary Marker Chromosome 15. *N. L. Hoppman-Chaney[1], D. B. Dawson[1], L. P. Nguyen[1], S. Sengupta[1], E. McPherson[2], K. Reynolds[2], G. Velagaleti[1]* 1) Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN; 2) Marshfield Clinic, Marshfield, WI.

Deletions and imprinting abnormalities of the Prader-Willi Angelman Syndrome Critical Region (PWASCR), located at chromosome 15q11.2-q13, typically result in either Prader-Willi or Angelman Syndrome. Recently it has been recognized that extra copies of the PWASCR, derived from intrachromosomal duplication or generation of a supernumerary marker chromosome (SMC), have detrimental phenotypic effects as well. Hexasomy for the PWASCR is extremely rare as only 6 cases have been described to date; 2 of which are due to a tricentric SMC 15 with 4 copies of the PWASCR. These patients typically have a broad range of phenotypic findings, including hypotonia, seizures, developmental delay and mental retardation. We identified a 13 year old female patient referred for developmental delay and seizure disorder with a tricentric SMC 15 through routine G-banding chromosome studies. C-banding and fluorescent in situ hybridization (FISH) using probes D15S10, SNRPN, and D15Z1 confirmed the presence of three chromosome 15 centromeres as well as 4 copies of the PWASCR on the SMC in approximately 60% of interphase cells, in addition to 2 copies on the normal chromosome 15s. Methylation-sensitive multiplex ligation-dependent PCR amplification using a commercially available kit revealed that the extra copies of the PWASCR contained on the marker chromosome bear a methylation pattern similar to a normal maternal chromosome, implying maternal inheritance. In conclusion, these results confirm the presence of 4 copies of the critical region on the SMC, resulting in hexasomy for the PWASCR. The extra copies of the critical region are likely maternally imprinted, consistent with the patients phenotype as paternal inheritance of such a marker chromosome is believed to be benign. The patient has some phenotypic overlap with Prader-Willi Syndrome including hypotonia, mild short stature, and obesity. Microsatellite marker testing is currently underway to confirm maternal inheritance of the SMC.

46,XY,i(18)(q10)/46,XY,del(18)(p11.1) mosaicism in an infant with mild congenital anomalies. *S. Kantarci[1,2], J. Picker[2,3], A. B. S. Giersch[2,4], S. Lincoln[3], M. L. Gorman[1]* 1) Beth Israel Deaconess Medical Center, Boston, MA; 2) Harvard Medical School, Boston, MA; 3) Children's Hospital, Boston, MA; 4) Brigham and Women's Hospital, Boston, MA.

A newborn male infant was referred to the Genetics Clinic because of hypotonia, respiratory distress, heart murmur, and minor dysmorphic features. The pregnancy and spontaneous vaginal delivery were uneventful. Review at 4 months of age was normal for hearing, vision and systemic review. Developmentally there were no concerns; he was interactive, alert, aware and vocalizing. He fed well, and was generally happy. Physical examination at 4 months of age revealed atrial and ventricular septal defects and borderline aortic arch hypoplasia. Mild nonspecific enlargement of the liver and spleen were detected by abdominal ultrasound. Head circumference was 25th centile, height 10th centile and weight 25th -50th centile. Forehead was mildly sloped, nose flattened and ears had everted prominent lobes with posterior creases and notched and flattened upper helices.The upper limbs had rhizomelic shortening, but the lower limbs were normal. There were no other dysmorphic features and neurological exam was entirely normal. Cytogenetics of peripheral blood showed a mosaic karyotype: 46,XY,i(18)(q10)[14]/46,XY,del(18)(p11.1)[6]. To our knowledge, only five cases with mosaic i(18q)/del(18p) have been reported. Clinical findings of this mosaic constitutional abnormality are not yet fully delineated due to the limited number of cases and therapeutic abortion of the affected fetuses. Clinical findings of del(18p) include growth deficiency, variable degrees of mental retardation, muscular hypotonia, speech delay, craniofacial dysmorphism (holoprosencephaly, broad face, ptosis, strabismus, hypertelorism, low nasal bridge, micrognathia, large protruding ears), hypopituitarism, and limb, genital and cardiac abnormalities. Findings of i(18q) are characteristic of both trisomy 18 and del(18p), or characteristic of trisomy 18 alone. Our case, similar to the previously reported five mosaic cases, show primarily the findings of del(18p) rather than i(18q).

Ultrasound findings in 92 cases of trisomy 18. *B. E. Kamen[1], P. A. Romitti[2], S. R. Patil[3], V. Kancerla[2], K. Borowski[3,4], J. Yankowitz[4]* 1) College of Medicine, University of Iowa, Iowa City, IA; 2) Department of Epidemiology, College of Public Health; 3) Department of Pediatrics, College of Medicine; 4) Department of OB/GYN.

OBJECTIVE: Recognizing trisomy 18 (T18) is important to provide options and counseling to patients. We determined the percent of T18 fetuses that had abnormal prenatal ultrasound findings, the types of abnormalities and proportion of fetuses with each. STUDY DESIGN: In this retrospective chart review, all women with a pregnancy complicated by fetal T18 who had a prenatal ultrasound examination at our institution between July 1989 and March 2006 were included. Sonograms were reviewed, identifying anatomical, and cord, growth and amniotic fluid abnormalities. Average number of organ systems affected, number of abnormalities and number of patients with each abnormality were calculated. Cases that had no or mild anomalies were closely reviewed to ascertain risk factors for failure to detect serious sonographic pathology. RESULTS: 92 cases were identified. Patients were most commonly referred due to isolated defects (29.3%). The average gestation age at first ultrasound was 20.2 weeks and average maternal age was 32.2 years. Eighty four percent of all fetuses had one or more defects which increased to 85% if ultrasounds prior to 14 weeks were excluded. The central nervous system (65%) and cardiac system (61%) were the most commonly affected organs. The most common individual defects were VSDs (50%), postured hands (37% each) and choroid plexus cysts (36%). Of the 13 patients with no abnormalities after 14 weeks, four ultrasounds failured to visualize all anatomy, 3 were twin pregnancies with discordant growth, 3 had isolated CPCs and 3 had a normal ultrasound. Ten of these 13 women were 35 years of age or older. CONCLUSION: This largest study to date (based on a Medline search) shows that about 85% of fetuses with T18 have severe anomalies on prenatal ultrasound. Caution should be exercised in patient counseling following ultrasound when factors like advanced maternal age or abnormal serum screening are coupled with either failure to adequately visualize all anatomy or mild abnormalities are seen.

Exonic hepatic lipase variant associated with insulin resistance in the NHLBI Family Heart Study (FHS). *M. F. Feitosa[1], P. An[1], R. H. Myers[2], J. S. Pankow[3], M. A. Province[1], I. B. Borecki[1]* 1) Washington Univ, St. Louis, MO; 2) Boston Univ, Boston, MA; 3) Univ Minnesota, Minneapolis, MN.

The hepatic lipase gene (LIPC) plays a major role in lipoprotein metabolism and belongs to a network of genes implicated in dyslipidemia and diabetes pathogenesis. The homeostatic model assessment (HOMA-IR), which is calculated as a function of fasting glucose and insulin, has been used to quantify insulin resistance. We investigated whether HOMA was associated with LIPC variants in Caucasians from pedigree-based FHS data and also follow-up in a FHS genome wide association study (FHS GWAS) of 1000 largely unrelated subjects. First, we genotyped 19 tag-SNPs spanning ~137 kb including LIPC in 591 families (2238 subjects). A family-based association test (FBAT) showed significant association (p=0.0019, FDR-p=0.0360) in women between HOMA-IR and rs17190678, which is in linkage disequilibrium (LD) with variants within exon-5 of the gene (r2=0.60). Further, we genotyped 897 unrelated FHS GWAS subjects with 110 tag SNPs across 264 kb of LIPC. There was evidence of association between HOMA-IR with rs6083, a SNP within exon 5 of LIPC (p=0.00072, FDR-p=0.020, by using PLINK). The association was stronger after excluding diabetics or subjects taking medication for diabetes (rs6083: p=0.00037). Association was also found between HOMA-IR with SNPs in introns 4, 6, and 8 (rs17190650, rs7178362, and rs17269397, respectively; 0.000083p0.00053, 0.0092FDR-p 0.019). These four LIPC markers are in LD with each other (0.77LD0.97) based on the CEU HapMap data. The non-synonymous LIPC variant (rs8063; Ser215Asn) minor allele (frequency=0.37) is associated with higher HOMA-IR level, suggesting greater insulin resistance. To construct a proper replication sample, we excluded from the case-control sample all subjects in the pedigree discovery sample, leaving N=351 subjects. The association of HOMA-IR with the four LIPC markers remained (0.004p0.025), despite a 60% reduction in the sample size. This is the first report of association of LIPC Ser215Asn influencing insulin resistance. The current findings suggest that LIPC may be part of the suite of genes that link diabetes and dyslipidemia.

Gene and Pathway-Based Analysis Second Wave of Genome-wide Association Studies. *L. Luo[1], G. Peng[2], H. Siu[2], Y. Zhu[2], P. Hu[2], S. Hong[2], J. Zhao[3], X. Zhou[4], J. Reveille[4], C. Amos[5], L. Jin[2], M. Xiong[1]* 1) Human Genetics Center, University of Texas School of Public Health, Houston, TX 77030; 2) School of Life Science, Fudan University, Shanghai 200433 , China; 3) Department of Medicine, Emory University of School of Medicine, Atlanta, GA 30306; 4) Division of Rheumatology, Medical School, University of Texas of Texas Health Science Center at Houston, Houston, TX 77030; 5) Department of Epidemiology, University of Texas, M. D. Anderson Cancer Center, Houston, TX 77030.

Despite great success of GWA studies in identification of common genetic variants associated with complex diseases, the current GWA studies have focused on single SNP analysis. However, Single SNP analysis often identifies a number of most significant SNPs that account for only a small proportion of the genetic variants and offers limited understanding of complex diseases as an integrated whole. To overcome these limitations, we propose gene and pathway-based genome-wide association analysis as a new paradigm for GWA studies. As a proof of concept, we performed a comprehensive gene and pathway-based genome-wide association studies for eleven diseases in seven studies. We found that 42 significant genes and 45 pathways associated with rheumatoid arthritis in WTCCC studies which were replicated in NARAC&EIRA studies, 7 significant genes and 10 pathways associated with type 2 diabetes in WTCCC which were replicated in DGI and 82 significant genes and 30 pathways associated with Parkinson's Disease in NINDS Parkinsonism study which were replicated in NINDS Parkinson disease study. Our results showed that the proposed new paradigm for GWA studies not only identified the genes that include significant SNPs found by single SNP analysis, but also detected new genes in which each single SNP conferred small disease risk, but their joint actions were implicated in the development of diseases. The results also demonstrated that the new paradigm for GWA studies were able to identify biologically meaningful pathways associated with the diseases which were confirmed by gene-set rich analysis using gene expression data.

Comprehensive Analysis of miRNA, mRNA, Methylation and Genotype Data. *H. Dong[1,2], X. Fang[3,2], H. Siu[1], L. Luo[2], G. Peng[1], Y. Zhu[1], R. Chen[4], D. Wheeler[4], M. Xiong[2,1]* 1) Dept Genetics, School of Life Science, Fudan University, Shanghai 200433, China; 2) Human Genetics Center, University of Texas School of Public Health, Houston, TX 77225, USA; 3) School of mathematical sciences, Peking University, Beijing 100871, China; 4) Human Genome Sequencing Center, Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas 77030, USA.

With great progress in experimental technologies, during the past decade, in addition to transcription factors, novel gene regulatory mechanisms have been discovered. Both genomic and epigenetic alterations such as DNA variations, transcription factors, MicroRNAs (miRNAs) and Methylation play crucial roles in a variety of regulations. In order to decipher regulatory mechanisms we investigated 900,660 SNPs, expression profiles of 470 miRNA, 2, 994 Methylation and 11,860 genes in 145 shared brain cancer samples from TCGA project. We found that total 100,481 SNPs and 263 cis-SNPs which significantly regulated expressions of miRNA. On average, each miRNA was regulated by 1,441SNPs and 0.875 cis SNP (P-value < 5.6*10-8). We also found that miRNA significantly influenced total 409 pathways and that on average, each miRNA regulated 20 pathways. We discovered that miRNA significantly regulated expressions of 4265 genes among which there were 44 transcription factors. Some results were confirmed by other sources. We use network models as a framework and structural equations as an analytic tool to integrate analysis of various regulatory components and construct a unified network that connect all components of miRNA, mRNA, Methylation and SNPs. These results reveal novel and deep mechanisms of regulation and provide valuable information for future research in gene regulatory systems.

The G67E mutation in hMLH1 is associated with an unusual presentation of HNPCC. *V. Yu[1,2], E. Hoffman[3], S. Shanley[2], S. Payne[4], S. Fisher[4], R. Barnetson[6], I. Frayling[5], A. Barrett[7], A. Arden-Jones[2], M. Clyne[3], J. Offman[3], J. Virgo[3], R. Eeles[2,8]* 1) Faculty of Medicine, MRC Clinical Sciences Centre, London, London, United Kingdom; 2) Cancer Genetics Unit, Royal Marsden Hospital, Fulham Road, London, SW3 6JJ, UK; 3) MRC Genome Damage and Stability Centre University of Sussex Science Park Falmer BN1 9RQ; 4) North West Thames Regional Genetics Service, Northwick Park & St Marks Hospital, Watford Road, Harrow, London, HA1 3UJ, UK; 5) Institute of Medical Genetics, Cardiff University, School of Medicine, Heath Park, Cardiff, UK; 6) Colon Cancer Genetics Group, University of Edinburgh Cancer Research Centre and MRC Human Genetics Unit, Western General Hospital, Edinburgh, EH4 2XU, United Kingdom; 7) University of East Anglia Norwich NR4 7TJ United Kingdom; 8) Institute of Cancer Research, Brookes Lawley Building, 15 Cotswold Rd, Belmont, Sutton, Surrey, SM2 5NG, UK.

Mutations in the mismatch repair genes are associated with hereditary nonpolyposis colorectal cancer (HNPCC). Here we identify and characterize a new variant of hMLH1 that confers a loss-of-function mismatch repair (MMR) phenotype. The mutation changes the highly conserved G67 residue to a glutamate (G67E) and is reminiscent of the hMLH1-G67R allele, which is present in several HNPCC cohorts. hMLH1-G67R has previously been assessed to confer loss-of-function, and two functional assays suggest that the hMLH1-G67E protein fails to sustain normal MMR functions. In the first assay, hMLH1-G67E abolishes the proteins ability to interfere with MMR in yeast. In the second assay, mutation of the analogous residue in yMLH1 (yMLH1-G64E) causes a loss-of-function mutator phenotype similar to yMLH1-G64R. Despite these molecular similarities, family analysis shows that the cancer spectrum associated with hMLH1-G67E are atypical of HNPCC and different from those found in families carrying the hMLH1-G67R allele. This suggests hMLH1 may have different functions in certain tissues and/or that additional factors may modify the influence of hMLH1 in causing HNPCC.

Genome-wide association scans for secondary traits using case-control samples. *G. M. Monsees[1], P. Kraft[1,2]* 1) Dept of Epidemiology, Harvard School of Public Health, Boston, MA; 2) Dept of Biostatistics, Harvard School of Public Health, Boston, MA.

Genome-wide association studies (GWAS) require considerable investment, so researchers often study multiple traits collected on the same set of subjects to maximize return. This has lead many many GWAS to adopt a case-control design; however, improperly accounting for case-control ascertainment can lead to biased estimates of association between markers and secondary traits. We show that under the null hypothesis of no marker-secondary trait association, naïve analyses that ignore ascertainment or stratify on case-control status have proper Type I error rates except when both the marker and secondary trait are independently associated with disease risk. Under the alternative hypothesis, these methods are unbiased only when the secondary trait is not associated with disease risk. We also show that inverse-probability-of-sampling-weighted (IPW) regression provides unbiased estimates of marker-secondary trait association. We use simulation to quantify the Type I error, power and bias of naïve and IPW methods. IPW regression has appropriate Type I error in all situations we consider, but has lower power than naïve analyses. The bias for naïve analyses is small provided the marker is independent of disease risk. Considering the majority of tested markers in a GWAS are not associated with disease risk, naïve analyses provide valid tests of and nearly unbiased estimates of marker-secondary trait association. Care must taken when there is evidence that both the secondary trait and tested marker are associated with the primary disease, a situation we illustrate using an analysis of the relationship between a marker in FGFR2 and mammographic density in a breast cancer case-control sample.

A Disease Severity Scoring System for Type 1 Gaucher Disease. *S. vom Dahl[1], M. D. Cappellini[2], T. Cox[3], E. H. Giannini[4], G. A. Grabowski[4], W. L. Hwu[5], H. Mankin[6], A. M. Martins[7], C. Sawyer[8], N. Weinreb[9], M. Yeh[8], A. Zimran[10]*
1) St. Franziskus Hosp, Cologne, Germany; 2) Univ degli Studi di Milano, Milano, Italy; 3) Univ of Cambridge, Cambridge, UK; 4) Cincinnati Childrens Hosp Medical Center, Cincinnati, USA; 5) National Taiwan Univ Hosp, Taipei, Taiwan; 6) Mass General Hosp, Boston, USA; 7) Univ Federal de Sao Paulo, Sao Paulo, Brazil; 8) Genzyme Corp, Cambridge, USA; 9) Univ Res Foundation for Lysosomal Storage Disorders, Coral Springs, USA; 10) Shaare Zedek Medical Center, Jerusalem, Israel.

Introduction: A validated disease severity scoring system (DS3) for type 1 Gaucher disease (GD1) is needed to monitor progression and treatment response in individuals and to compare patient cohorts in clinical studies. Objective: To develop and test the reliability and validity of a DS3 to assess and monitor GD1 in adults. Methods: DS3 domains were established by an expert physician group using nominal group technique (NGT) consensus formation methodology. Items within domains were selected by Delphi survey of 32 physician experts in GD1. The expert group determined appropriate measurement techniques for each variable, and measurements were weighted considering how much morbidity and mortality each contributes to GD1. Twelve physicians with expertise in GD1 and no prior exposure to the DS3 rated content validity and feasibility and scored patient profiles using the DS3 and the Clinical Global Impression Severity Scale (CGI-S). Results: The DS3 includes bone, hematological, and visceral domains, which were populated with weighted measurements of GD1 signs and symptoms. Relevance, feasibility, and inter-rater reliability were assessed by content validity index (83%), feasibility index (92%), and intraclass correlation (0.89), respectively. Patient profile DS3 scores were highly correlated with CGI-S scores (R^2=0.90). Conclusions: The DS3 working model is feasible, relevant, and reliable for assessing disease severity in adults with GD1. DS3 scores highly correlate to those obtained by a validated severity scale (CGI-S), but are more specific to GD1 and responsive to change. Testing in larger patient cohorts and of additional aspects of reliability and validity will continue.

Rare mutation detection utilizing different Next Generation DNA sequencing platforms. *D. Smith[1], A. Huehls[2], B. Eckloff[3], S. Middha[4], Y. Asman[4], J. P. Kocher[4], M. Barker[5], P. Milos[6]* 1) Lab Med & Path/Exper Path, Mayo Clinic, Rochester, MN; 2) Molecular Pharmacology and Experimental Therapeutics, Mayo Clinic, Rochester, MN; 3) Advanced Genomics Technology Center, Mayo Clinic, Rochester, MN; 4) Health Sciences Research, Mayo Clinic, Rochester, MN; 5) Applied Biosystems, Foster City, CA; 6) Helicos Biosciences Corporation, Cambridge, MA 02139.

There are now multiple platforms available that have the capabilities of utilizing multiple parallel DNA sequencing to sequence millions of DNA templates. There are numerous applications of this novel technology including the ability to detect rare mutations from biological fluids. We decided to test three of the currently available platforms which produce short sequence reads to compare their ability to detect rare mutations: the Illumina Genome Analyzer, the ABI SOLID system and the Helioscope from Helicos. We chemically synthesized a wild type 58 base pair region from exon 5 of the p53 gene and cloned this into the bacterial vector pUC57. We also cloned four different mutations within this same region of the p53 gene. The fragments were then cloned into E. coli to take advantage of the fidelity of bacterial replication. Single clones with the correct insertions were then grown up to produce large quantities of intact plasmid for dilution experiments. We then made seven different mixtures of plasmids for deep sequencing. The first corresponded to 100% wild type sequence. The remaining six had different concentrations of all four different mutations ranging in concentration from 2% all the way down to 0.02%. While we started this experiment by cutting out a 200 base pair piece containing the synthetic p53 insertion we quickly realized that each of these sequencing platforms could easily completely deep sequence the entire 2710 bp pUC57 plasmid with the insertions. The resulting overlapping short sequence reads generated were then aligned to determine the ability of each of these three platforms to detect rare mutations and to determine what frequency of rare mutations could be detected with each platform. These technologies clearly have the ability to detect rare mutations and thus have tremendous clinical potential.

Genome-wide Pathway Analysis. *M. Xiong[1], L. Luo[1], G. Peng[2], Y. Zhu[2], C. Amos[3]* 1) Human Genetics Center, University of Texas School of Public Health, Houston, TX 77030; 2) School of Life Science, Fudan University, Shanghai 200433 , China; 3) Department of Epidemiology, University of Texas, M. D. Anderson Cancer Center, Houston, TX 77030.

To meet conceptual and technical challenge raised by GWA and take full advantage of the huge opportunities provided by GWA, we propose to develop gene and pathway-based genome-wide association studies as a new paradigm for genome-wide association studies of complex disease. One framework to test for association of genes with disease is to directly develop statistics for global testing association of genes with disease. Another framework is to combine p-values of individual SNP tests. In this report, we will focus on combining individual association tests of SNPs because this approach has close relationships with the traditional single SNP-based association analysis. The popular method for combining individual evidence is meta-analysis. Widely used meta-analysis is to combine independent P-values of individual tests. However, in general, correlations among p-values of SNPs within the gene exist due to LD among SNPs. Correlations among SNPs will invalidate the methods for combining independent p-values. Therefore, to perform gene and pathway-based GWA studies, we need to develop methods for combining dependent p-values which take correlations among SNPs into account. We propose three statistics for combining dependent P-values: Linear Combination Test, quadratic test and Decorrelation test. We calculate type 1 error of the developed statistics and study influence of the correlations among SNPs or genes on the test statistics. We assembly about 1,000 pathways from KEGG and BIOCART pathway database. The proposed statistics have been applied to genome-wide association studies of five diseases. The preliminary results show that the proposed methods dramatically increase the power to identify the genes and pathway associated with the diseases and improve the ability to replicate the association finding. The results also demonstrate that the pathway-based association analysis makes interpretation of the results and unravel the mechanism of the diseases much easier.

Three Ways of Genome-wide Pathway Analysis. *X. Zhou[1], H. Xiong[2], L. Luo[3], F. Arnett[1], M. Xiong[3]* 1) Division of Rheumatology, Medical School, University of Texas of Texas Health Science Center at Houston, Houston, TX 77030; 2) Department of Computer Science,Texas A&M University,College Station, TX 77843; 3) Human Genetics Center, University of Texas School of Public Health, Houston, TX 77030.

We assume that three types of data: genome-wide time course gene expression data, genome-wide genotype data and clinical endpoint of phenotypes are available. Traditional genome-wide association (GWA) studies is to use a SNP as a basic unit of association and to test association of a single SNP at a time. Similar to gene set enrichment analysis of gene expression data, we propose a pathway-based GWA studies that use a pathway as a basic unit of association and test association of a pathway with the disease. We also propose how to use continuous state-space model to connect cis and trans eQTLs to significantly differentially expressed pathway. We propose a general procedure for jointly performing three ways of genome-wide association analysis of complex diseases, which uses pathway analysis as a unified strategy for conducting genome-wide association studies and network model as a general framework for integration of genotype, gene expression and phenotype data. The procedure consists of two steps. First step is to perform genome-wide pathway analysis of gene expression, pathway-based GWA studies of disease and pathway-based genetic analysis of time course gene expression using state-space model. Second step is to use network model and meta-analysis to jointly conduct three ways of genome-wide pathway analysis, which will lead to deciphering path from genomic information through gene expression to clinical endpoints of disease. The proposed methods have been successfully applied to real systems sclerosis data.

Identification of novel CDH1 germline mutations in diffuse gastric cancer and lobular breast cancer. *W. Zeng[1], K. Gonzalez[1], M. Parra[1], H. Dominguez[1], C. Fong[1], S. Sommer[2], J. S. Saldivar[1]* 1) Molecular Diagnostic Laboratory, City of Hope National Me, Duarte, CA; 2) Department of Molecular Genetics, City of Hope National Medical Center, Duarte, CA.

Germline mutations in the CDH1 gene which encodes E-cadherin, a cell-cell adhesion protein, have been identified in hereditary diffuse gastric cancer. Associations between CDH1 germline mutations and both lobular breast cancer and signet ring carcinoma of the colon have been reported in HDGC families. Since offering CDH1 full gene sequencing for clinical testing, 93 diffuse gastric cancer families have been analyzed in our laboratory. All of the 16 coding exons and associated intron junctions of the CDH1 gene were directly sequenced using ABI 3730 sequencer. A total of 24 germline mutations were found in 93 families, giving a detection rate of about 26%. Of the 24 mutations identified, eighteen were in families that had at least two or more cases of gastric cancer in a family, with at least one diffuse gastric cancer diagnosed before age 50 years (18/24; 75%); the other six mutations were distributed equally in six families with two mutations each found in patients meeting the 2nd, 3rd, and 4th revised IGCLC criteria1. Twelve of these mutations identified were novel: 4 splicing mutations, 7 truncating mutations (4 nonsense, 1 deletion, and 2 insertions), 1 missense/splicing mutation. Among those probands who tested positive for a mutation, one had bladder cancer at 52 years of age followed by diffuse gastric cancer diagnosed at 54 yrs; another proband had Hodgkin's disease at 14yrs followed by a diagnosis of diffuse gastric cancer at 26 years. The current data identified additional 13 novel mutations in the CDH1 gene. The occurrence of extra-gastric tumors associated with CDH1 mutations warrants further investigation. 1. Brooks-Wilson et al. J Med Genet. 2004; 41: 508-17.

Continuous State-Space Model for Genetic Analysis of Time-Course Gene Expression Data. *H. Xiong[1], L. Luo[2], F. Arnett[3], X. Zhou[3], M. Xiong[2]* 1) Department of Computer Science,Texas A&M University,College Station, TX 77843; 2) Human Genetics Center, University of Texas School of Public Health, Houston, TX 77030; 3) Division of Rheumatology, Medical School, University of Texas of Texas Health Science Center at Houston, Houston, TX 77030.

Enormous difficulties arise from analyzing large temporal observations in gene regulation and signal transduction networks. Traditional quantitative genetics has primarily studied traits that are measured at a specified location or time. DNA is assigned a static role. The traits are investigated as isolated and static variables. Therefore, they have limited power to untangle the complicated biological networks and to decipher the mystery of the paths that connect genotypes and phenotypes. In this report, we propose to use a continuous state-space model for genetic studies of gene regulation and signal transduction networks, Specifically, we model time-course gene expression data of genetic networks as a dynamic biological system which will finally lead to stochastic differential-algebraic equations. The concept of a dynamic system that originally arose from the Newtonian mechanics is now widely used as a basic framework for scientific research. Dynamic models not only focus on functional values themselves, but also focus on the rates of changes of functions. Dynamic models allow us to simultaneously model both the function itself and its derivatives. We propose to use EM algorithms to estimate the parameters of stochastic differential-algebraic equations which are based on continuous Kalman filter. We then develop statistics to test association of SNPs with gene regulation and signal transduction networks. We use both simulation and real time course gene expression and genome-wide genotype data of systems sclerosis to validate the models. Preliminary results show that continuous state-space models are powerful tool for investigation of genetics of time course gene expression data and other longitudinal genetic studies of complex diseases.

Mapping a novel locus for microcephaly, learning disability, and congenital cardiovascular and renal anomalies.

C. L. Beaulieu[1], K. M. Boycott[2], D. R. McLeod[1], A. M. Innes[1], J. S. Parboosingh[1] 1) Dept of Medical Genetics, University of Calgary, Calgary, Alberta, Canada; 2) Dept of Genetics, Children's Hospital of Eastern Ontario, Ottawa, Ontario, Canada.

The Hutterites are a genetically isolated Anabaptist group living on the North American prairies; their population numbers over 40,000, the majority of whom are descendants of 89 founders. An autosomal recessive neurodevelopmental disorder was identified in four patients from two consanguineous Hutterite families. To our knowledge the clinical presentation is unique and to date undescribed. The patients have distinctive facial features, congenital malformations of the heart and genitourinary system, borderline microcephaly, and nonverbal learning disability. The facial features include very tall forehead with high anterior hairline, deep-set eyes with short palpebral fissures, long nose with overhanging columnella, and full lips. The spectrum of congenital malformations includes ventricular septal defect, patent ductus arteriosis, and horseshoe kidney. Karyotype and baseline metabolic studies were normal.

An identity-by-descent mapping approach was used to identify the locus for this disorder. The patients were genotyped using a 50K-SNP microarray which identified a single, strong homozygous region on 16p13.3 which was shared between all of the patients. To confirm and refine the boundaries of this region, microsatellite markers were genotyped for the patients, the parents, and the available unaffected siblings. The disease locus was refined to a region of 5.1Mb containing 160 known or predicted genes. No other recessive disorders with similar clinical features are currently mapped to this region. Prioritization of genes within the region through data searching and microarray expression analysis is underway, and no mutations have been identified by sequence analysis of 40 genes.

Identification of Men with a genetic predisposition to ProstAte Cancer: Targeted screening of *BRCA1/2* mutation carriers and controls. The IMPACT study. Pilot results show a high frequency of positive biopsy. *A. Mitra[1], E. Bancroft[2], R. Eeles[1]* 1) Institute of Cancer Research, London, UK; 2) Royal Marsden Hospital NHS Foundation Trust, London, UK.

IMPACT, an international collaborative study is a targeted prostate cancer (PrCa) screening study of men with a known germline mutation which is thought to predispose to the disease. Male *BRCA1/2* mutation carriers and a control group who have tested negative for a mutation known to be present in their family, are screened annually with a serum PSA test. The threshold for prostate biopsy is PSA > 3ng/ml. The study is designed to recruit men over a period of 5 years and has been running for 31 months. The pilot data for 237 men aged 40-69 years (median age 54 years) from the first screening round has been collected. In total, 6 men have had a PrCa diagnosed and 2 men are awaiting biopsy. The European Randomised Study for Prostate Cancer (ERSPC) PSA threshold is PSA >= 3ng/ml. The number of men with a PSA>= 3ng/ml in IMPACT is 17/237 (7.1%) compared with 2048/10191 (20.0%) in the ERSPC. Of note, the PSA assay in the ERSPC produces values that are ~20% higher than the assay used in IMPACT, but this would still not account for this difference. The median age of men in IMPACT is 54 years compared with 66 years in the ERSPC. This may account for the lower biopsy rate. However, in IMPACT, of those biopsied at this threshold, 6/13 (46%) are positive for PrCa whereas in ERSPC this value was 541/1850 (29%). The PrCas that develop in mutation carriers have a higher proportion of Gleason pattern 4 or more advanced disease compared with non-carriers. Targeted screening based on *BRCA1/2* germline mutation is feasible and the biopsy rate is acceptable. The proportion of positive biopsies is much higher than in the population based screening studies and the disease is aggressive. These pilot data indicate that the main IMPACT study should proceed and that targeted screening based on genotype has a high yield of positive biopsy.

Improved clinical outcome in a Pompe disease patient increased from 20 to 40 mg/kg Myozyme every 2 weeks. *K. Kim[1,2], S. Widera[1], B. Burton[1,2]* 1) Division of Genetics, Children's Memorial Hospital, Chicago, IL; 2) Department of Pediatrics, Feinberg School of Medicine, Northwestern University, Chicago, IL.

We report on a 4 year old boy, RC, with Pompe disease, who exhibited significant clinical and biochemical improvement following an increase in biweekly Myozyme (alglucosidase alfa) dose from 20 mg/kg to 40 mg/kg. RC was diagnosed with Pompe disease when increased glycogen content was noted on muscle biopsy at age 9 mos and deficiency of acid -glucosidase activity was demonstrated in multiple tissues. He was felt to have late-onset infantile type based on clinical symptoms and *GAA* mutation results. Enzyme replacement therapy (ERT) with biweekly infusions of Myozyme at 20 mg/kg was initiated at age 20 mos. At the start of ERT, RC had generalized hypotonia, myopathic facies, calf pseudohypertrophy, and motor delay. He was crawling, cruising and pulling to stand with assistance. Echo and ECG were normal. Apnea was noted on polysomnogram. Myozyme infusions were well tolerated with no infusion associated reactions (IARs) observed. After 10 mos of therapy, RC could take a few independent steps but continued to exhibit an exaggerated broad-based and lordotic gait with side to side steps. No additional gains in motor milestones were subsequently observed. After 18 mos on ERT, Myozyme dose was increased to 30 mg/kg for 3 mos and then further increased to 40 mg/kg after infusions were well tolerated and parents reported increased activity level and endurance. On exam after 6 mos of biweekly infusions of Myozyme at 40 mg/kg, RC was more active and vigorous with improved muscle strength and tone and a dramatically improved gait. His facial appearance was more animated. No IARs and no change in antibody titers were observed at the increased dose. A decrease in urine hexose tetrasaccharide levels was observed. Based on our experience, we conclude that improvement of skeletal muscle symptoms can be observed in some patients with an increase in Myozyme dose. Higher doses can be well tolerated in some young patients without observed IARs. Therefore, Myozyme dosing should be patient specific and higher doses should be considered and further investigated to achieve an improved outcome.

The male-determining gene SRY is a hybrid of DGCR8 and SOX3, and is regulated by the transcription factor CP2. *Y. Sato, T. Shinka, K. Sakamoto, Y. Nakahori* Tokushima Univ, Tokushima, Japan.

In mammals, sex is determined by the presence or absence of the Y chromosome that bears a male-dominant sex-determining gene SRY, which switches the differentiation of gonads into male testes. The molecular signaling mechanism turning on the switch, however, has remained unclear for 18 years since the identification of the gene. Here, we describe how this gene emerged and started to work. From amino acid homology, we realized that SRY is a hybrid gene between a portion of the first exon of DiGeorge syndrome critical region gene 8 (DGCR8) and the high-mobility group (HMG) box of SRY box-3 (SOX3) gene. We pinpointed the regulatory sequence in the SRY promotor region by searching for a common motif shared with DGCR8 mRNA. From the motif search between DGCR8 mRNA and the SRY upstream sequence, we found that the TFCP2 binding motif is present in both. TFCP2 overexpression led to up-regulation of SRY mRNA expression, and TFCP2 suppression by RNA interference (RNAi) significantly reduced SRY mRNA expression. Furthermore, electrophoretic mobility shift assay (EMSA) demonstrated that TFCP2 acts as a regulator by directly binding to the SRY promoter. We conclude that SRY is a hybrid gene composed of two genes, DGCR8 and SOX3; and TFCP2 is an essential transcription factor for SRY expression regulation.

Correlation between *EGFR* mutations and the effectiveness of gefitinib in patients with lung adenocarcinoma. *M. Takasu[1], H. Nishioka[1], T. Mawatari[1], E. Iwamoto[1], S. Kondo[1], H. Yamaguchi[2], K. Nakatomi[2], Y. Nakamura[2], S. Kohno[2], K. Tsukamoto[1]* 1) Dept Pharmacotherapeutics, Nagasaki Univ Grad Sch Biomed Sci; 2) Second Dept Inter Med, Nagasaki Univ Sch Med.

Objective: Somatic mutations of the epidermal growth factor receptor (*EGFR*) show dramatic clinical and radiographic response to the EGFR tyrosine kinase inhibitors, gefitinib, against patients with non-small cell lung cancer. There are hot-spot mutations of *EGFR*, such as 9- to 18-bp deletion in exon 19 and L858R point mutation in exon 21. The aim of this study was to evaluate correlation between *EGFR* mutations and the effectiveness of gefitinib in Japanese patients with lung adenocarcinoma. Methods: All of 46 samples (11 samples of fresh frozen surgical specimens, 14 paraffin-embedded surgical specimens, 10 transbronchial lung biopsy (TBLB) specimens, and 11 pleural fluid) from Japanese patients with lung adenocarcinoma, who received gefitinib treatment in the Nagasaki University Hospital or other hospitals in Nagasaki were examined the deletion of exon 19 and L858R of exon 21 of *EGFR* using mutant-enriched PCR assay. Subsequently, the outcomes of patients with or without *EGFR* mutations were compared with the effectiveness of gefitinib. Results: *EGFR* mutations (16 deletions and 14 L858R mutations) were detected in 23 patients (50%) out of all 46 patients. Response rate to gefitinib was 95.8% in patients with *EGFR* mutations, whereas 36.4% in those without mutations. The sensitivity and specificity of this analysis were estimated at 74.2% and 93.3%, respectively. The frequency of false-negative samples were higher in TBLB or pleural fluid than that in fresh frozen and paraffin-embedded surgical specimens. Conclusion: This study showed a significant clinical benefit of gefitinib in patients with *EGFR* mutations and indicated the availability of *EGFR* mutation analysis by mutant-enriched PCR assay. However, further studies are needed to establish the DNA-based diagnosis combining other gene mutation analysis and microdissection technique, in order to screen patients of high chemosensitivity to gefitinib and to decrease in the false-negative rate.

Identification of the disease gene susceptible to the progression of primary biliary cirrhosis in Japanese patients.

N. Iio[1], T. Inamine[1], S. Higa[1], F. Noguchi[1], S. Kondo[1], M. Nakamura[2], H. Ishibashi[2], K. Omagari[3], K. Tsukamoto[1] 1) Dept Pharmacotherapeutics, Nagasaki Univ Grad Sch Biomed Sci; 2) Clin Res Center, Natl Hosp Organi Nagasaki Med Center; 3) Dept Nutr, Siebold Univ Nagasaki.

Objective: Primary biliary cirrhosis (PBC) is a chronic and slowly-progressing autoimmune liver disease and is a multifactorial disease. In order to identify the unknown genetic determinants of onset, the disease severity, and progression of PBC, we examined an association of single nucleotide polymorphisms (SNPs) in the retinoid X receptor beta (*RXR*) gene as a candidate gene, because RXR is a nuclear receptor, which regulates expression of hepatobiliary transports and enzymes involved in bile acid metabolism and detoxification in the liver. Methods: The study subjects comprised 148 Japanese patients with PBC and 150 age- and gender-matched healthy control subjects. Patients were classified into three different clinical stages (I, II, and III) based on the findings of liver biopsy and clinical manifestations. Clinical stages I + II were defined as non-jaundice stage, whereas stage III was defined as jaundice stage. The two SNPs in *RXR*, rs2076310 and rs2072915, were detected by PCR-restriction fragment length polymorphism. Subsequently haplotypes were constructed from these SNPs. The frequencies of alleles, haplotypes, and diplotypes were compared between PBC patients and control subjects, as well as between subgroups of PBC patients by logistic regression analysis using SPSS 15. Results: Logistic regression analyses revealed that a haplotype, Hap 3, and a diplotype, Hap 1/Hap 3, in *RXR* were significantly increased in jaundice-stage PBC patients compared with nonjaundice-stage PBC patients [$P = 0.0004$, odds ratio (OR) = 6.96 and $P = 0.0002$, OR = 13.29, respectively]. Conclusion: The present study is the first report demonstrating the association between the haplotype and diplotype of *RXR* and the susceptibility to severe progression of PBC. Therefore, *RXR* is a genetic determinant of the progression of PBC, and could potentially be applied to DNA-based diagnosis in Japanese patients with PBC as a strong genetic biomarker for predicting the progression and prognosis of PBC.

Sequential change of gene expression profiles in the liver of Long-Evans cinnamon rats at different stages of developing hepatitis. *J. Kim[1], J. H. Kim[1], J. Y. ParK[1], G. Kim[1, 2], H. Yoo[1, 2]* 1) Genome Reserch Center , Asan Medical Center, Seoul, Korea; 2) Medical Genetic Clinic, Department of Pediatrics, Asan Medical Center, University of Ulsan, Seoul, Korea.

Wilson disease (WD), an autosomal recessive disorder of copper transport, is one of the most common inherited metabolic disorders in Korea. The Long-Evans cinnamon (LEC) rat, a natural animal model for Wilson disease caused by the mutation in the Atp7b gene homologous to the human ATP7B gene, shows similar clinical features as human Wilson disease including extensive copper accumulation in the liver with hepatitis and decreased serum ceruloplasmin level. This study was undertaken to investigate the sequential change of gene expression profiles in the liver of Long-Evans cinnamon rats at different stages of developing hepatitis. Total RNA was isolated from liver tissues of 9 LEC (each 3 animals per 6, 12, 24 weeks of age) and 9 wild type Long-Evans agouti (LEA) rats (each 3 animals per 6, 12, 24 weeks of age). Subsequently, total RNA was reverse-transcribed to cDNA using a T7 oligo(dT) primer. Second-strand cDNA was synthesized, in vitro transcribed, and labeled with biotin-NTP. Labeled cRNA samples were hybridized to each Rat-12 expression bead (Illumina, Inc., San Diego, USA). Detection of array signal was carried out using Amersham fluorolink streptavidin-Cy3 (GE Healthcare Bio-Sciences, Little Chalfont, UK) following the bead array manual. Arrays were scanned with an Illumina bead array Reader confocal scanner. Array data export processing and analysis was performed using Illumina BeadStudio. The results show that 278 genes turned out to be significantly differentially expressed among 22,517 target genes. At the age of 6 weeks, 23 were up-regulated and 29 down-regulated. At the age of 12 weeks, 68 were up-regulated and 33 down-regulated. At the age of 24 weeks, 87 were up-regulated and 38 down-regulated. During the progression of liver disease of LEC rats, the genes of molecular function related to oxidative stress, inflammation and apoptosis were up-regulated while the genes related to ribosomal protein, cytochrome oxidase and solute carrier family proteins tended to be down-regulated.

Polymorphisms of *iNOS* are associated with anti-tuberculosis drug-induced hepatotoxicity in Japanese patients with tuberculosis. *T. Mawatari[1], S. Fujie[1], M. Yosida[1], N. Higuchi[2], K. Tsukamoto[1]* 1) Dept Phrmacotherapeutics, Nagasaki Univ Grad Sch Biomed Sci; 2) Dept Pharmacy, Nagasaki Univ Hosp Med Dent.

Objective: Tuberculosis (TB) is a re-emerging infectious disease. TB care involves serious problems including the occurrence of adverse effects of anti-TB drugs. Our focus of interest in this study was hepatotoxicity, because it is the major and severe adverse effect. Recent studies suggest that hepatotoxicity is induced through reactive oxygen and nitrogen species by acetaminophen in mice. Thus, the aim of this study is to investigate whether polymorphisms of the inducible nitric oxide synthase (*iNOS*) gene, which is induced by reactive oxygen species and generates reactive nitrogen species, is associated with anti-TB drug-induced hepatotoxicity in Japanese patients with pulmonary TB.**Methods**: The study subjects comprised 100 Japanese patients with new onset of pulmonary TB treated at least with isoniazid and rifampicin-containing regimen for six or nine months. Nine single nucleotide polymorphisms (SNPs) in *iNOS* were determined by PCR-restriction fragment length polymorphism and PCR-direct DNA sequencing methods. Subsequently, haplotypes were constructed from the two SNPs, rs11080344 and rs3794756, because of the significant association of these SNPs with hepatotoxicity. The frequencies of alleles, genotypes, haplotypes, and diplotypes were compared between TB patients with and without hepatotoxicity.**Results**: Logistic regression analyses revealed that the frequency of a C/C genotype at the rs11080344 site in *iNOS* were significantly higher in TB patients with hepatotoxicity than those without it [$P = 0.044$, odds ratio (OR) = 2.87]. Moreover, a haplotype, Hap 1, and its homozygous diplotype, Hap 1/Hap 1, were significantly increased in TB patients with hepatotoxicity compared with those without it ($P = 0.0463$, OR = 2.098 and $P = 0.0086$, OR = 5.357, respectively).**Conclusion**: The present study is the first report demonstrating the association between *iNOS* polymorphisms and the susceptibility to anti-TB drug-induced hepatotoxicity. Therefore, the Hap 1/Hap1 diplotype in *iNOS* could be useful as a new biomarker for predicting anti-TB drug-induced hepatotoxicity.

Identification of novel mutations and the common mutation in the human *NOTCH3* gene of Korean patients with cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL). *G. Kim[1], J. Lee[1], I. Lee[1], J. Park[2], S. Choi[1], H. Yoo[1,2,3]* 1) Med Genetics Clinic & Lab, Asan Medical Center, Seoul, Korea; 2) Genome Research Center for Birth Defects & Genetic Disorders, Asan Medical Center, Seoul, Korea; 3) Department of Pediatrics, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea.

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is a small vessel disease of the brain, which is characterized by recurrent subcortical ischemic attacks, stepwise or progressive cognitive decline, and white-matter abnormalities on brain magnetic resonance images (MRI). The disease is caused by mutations of the NOTCH3 gene, which encodes a transmembrane receptor expressed in vascular smooth muscle cells. It has been reported that the mutations of NOTCH3 were mostly found in EGF-like domain 2-8 of NOTCH3 protein, which corresponded to exon 2 through 6 of the gene. To characterize molecular defects of the NOTCH3 gene in Korea patients with clinical diagnosis of CADASIL, we screened 840 unrelated patients with stroke, cerebral infarction, and/or vascular leukoencephalopathy by PCR-directed sequence analysis of hot spots on NOTCH3 gene using genomic DNA from peripheral blood leukocytes. We identified 27 distinct nucleotide variations in 130 out of 840 unrelated patients tested, including 22 cystein involved variations, four non-cystein involved variations (R75P, R75Q, P167S, L989R) and a indel mutation (p.Asp353_Cys355delins(8)). Ten novel mutations were identified (p.Asp353_Cys355delins(8), C65Y, R75Q, P167S, C285F, C291S, S414C, C606R, C971S, L989R). The R544C mutation was the most common mutation, which was identified in 71 out of 130 patients. In conclusion, we have identified 27 distinct mutations including 10 novel mutations of the NOTCH3 gene. The R544C accounts for 54.6% of disease alleles in Korean patients with CADASIL. Genetic testing should be considered as a confirmatory diagnostic test in clinically ambiguous cases.

Two cases with methionine adenosyl transferase deficiency presented with isolated hypermethioninemia on neonatal screening test. *H. Yoo, J. Ko, I. Lee, C. Cheon, G. Kim* Medical Genetics Clinic & Laboratory, Dept Pediatrics, Asan Medical Ctr, University of Ulsan College of Medicine, Seoul, Korea.

Neonatal screening for homocystinuria by measuring blood spot methionine concentrations is a common medical practice in worldwide. However, hypermethioninemia without homocystinuria (isolated hypermethioninemia) is also detected, and there is a possibility to be mistaken for homocystinuria. The conversion of methionine to S-adenosylmethionine by methionine adenosyl transferase (MAT) is the major pathway of methionine metabolism. Isolated hypermethioninemia is a clinically benign metabolic disorder associated with MAT I/III deficiency in liver due to the MAT1A gene mutation. Clinical manifestations are variable and poorly understood, and treatment for isolated hypermethioninemia remains controversial. We diagnosed two cases of isolated hypermethioninemia using the amino acid analysis and MAT1A gene analysis in newborns with increased methionine level detected by neonatal screening test for homocystinuria. They had no clinical symptom except increased level of blood spot methionine (8.0 and 4.0 mg/dL) on repeated screening tests. Serum amino acid quantitative analysis by HPLC was performed at 25 and 17 days old. Serum methionine (821 and 748 mol/L) and homocysteine (22.4 and 20.3 mol/L) levels were elevated. However, homocystine was not detected and cystine levels were normal. Low methionine diet was started, and analysis of the CBS gene (for homocystinuria) and the MAT1A gene (for MAT deficiency) was performed. We could not find any mutation in the CBS gene. However, MAT1A gene analysis revealed that they were compound heterozygote (p.Y92H/p.R299C, p.K97N/p.R199C). Three mutations are novel except p.R199C. They are 9 and 6 months old now, and develop normally without any treatment. Although serum methionine levels were persistently elevated during the follow-up period, serum homocysteine level has been normalized. Further studies for the natural history of isolated hypermethioninemia are necessary for reducing unwarranted anxiety of the parents and the health care professionals.

Combined Individual- and Family-level Genetic Association Analyses that Adjust for Population Stratification Bias. *L. Mirea[1,2], L. Sun[1,3], J. E. Stafford[1,3], S. B. Bull[1,2]* 1) Public Health Sciences, Univ of Toronto, Canada; 2) Samuel Lunenfeld Research Inst, Toronto, Canada; 3) Statistics, Univ of Toronto, Canada.

Population stratification bias (PSB) remains a serious concern in genetic association studies employing independent individuals. In the absence of empirical data regarding population structure, family-based designs can provide robust estimates, but association tests are less powerful due to uninformative allele transmissions. When both individual- (IA) and family-level (FA) analyses can be performed, the comparison between IA vs. FA has been used as a test for PSB and a basis for reporting either IA or FA results (Abecasis et al. 2000 *Am J Hum Genet* 66: 279-292), or pooling IA from case-control and FA from trio data (Chen and Lin, 2008 *Genet Epidemiol* early view). As an alternative, we derive and evaluate a novel test statistic Z_w that combines IA and FA using weights based on the observed evidence for PSB. To illustrate, for a quantitative trait measured in single individuals and trio offspring, the most efficient association analysis (IA_{tot}) employs all independent probands ignoring parental genotypes. Evidence for PSB is provided by the p-value (p_{EQ}) testing equality between orthogonal IA_o and FA_o components derived from analyses restricted to trios. We compute B_w as a weighted linear combination of IA_{tot} and FA_o estimates using weights p_{EQ} and (1- p_{EQ}), respectively. The test statistic Z_w is obtained by standardizing B_w under the null hypothesis of no association employing a robust covariance matrix, with significance evaluated assuming an asymptotic N(0,1) distribution. Using simulations, we show that for a variety of scenarios with and without PSB, the type I error of Z_w is always closer to nominal than that of previous approaches reporting either IA_{tot} or FA_o. In the absence of PSB, Z_w achieves greater power than FA_o, thus establishing Z_w as the best compromise between type I error control and power. A similar Z_w statistic can be computed for case-control and trio data. The adjustment for PSB is SNP-specific and depends on the power to detect PSB, and can be used in conjunction with other methods for correcting PSB in candidate gene or genome-wide studies.

Niemann-Pick type C disease (NP-C) is a considerable diagnosis in juvenile and adult-onset psychiatric disorders.

P. Bauer[1,2], T. Boettcher[2,3], W. Meyer[4], P. Martus[5], S. Weiss[2], R. Oheim[2], K. Heinze[2], M. Wittstock[2], A. Rolfs[2] 1) Dept Medical Genetics, Univ Tubingen, Tubingen, Germany; 2) Neurobiology Laboratory, Dept of Neurology, Univ Rostock, Rostock, Germany; 3) Dept of Neurology, Klinikum Neubrandenburg, Neubrandenburg, Germany; 4) Queen Mary Univ London, St. Bartholomew's and the Royal London School of Medicine, London, UK; 5) Institute of Biometry and Medical Epidemiology, Charité Berlin, Berlin, Germany.

Background: Niemann-Pick type C (NP-C) disease is an autosomal recessive neurovisceral storage disorder characterised by an intracellular trafficking deficit for cholesterol and other complex lipids. A wide spectrum of clinical symptoms includes hepatic and pulmonary disease and neurodegeneration with epilepsy, ataxia and neuropsychiatric disorders. The disorder is caused either by mutations in the NPC1 or the NPC2 gene. Usually, the first clinical symptoms of NP-C disease appear between ages of 4 and 10 years. However, there is raising evidence that the percentage of adult manifestations in NP-C disease is more frequent than up to now presumed. Objective: In order to assess NP-C frequency, genotypes and phenotypes, we completely sequenced the NPC1- and NPC2-gene in 189 adult Caucasian patients (aged between 15 and 51 years) hospitalized in psychiatric or neurological institutions diagnosed as having psychiatric diseases in combination with neurological symptoms, like epilepsy, ataxia, dystonia or dementia. ,. Results: In 19 (10%) of these patients, two pathogenic NPC1-mutations were identified, while in 7 patients only one mutation was detectable. Two pathogenic NPC2 mutations were observed in 3 patients which correspond to about 15% of all NPC disease cases. Conclusion: The high frequent of NPC disease in adult patients with neuropsychiatric symptoms makes it necessary to improve on one side the knowledge of this disease among neurologists and psychiatrists but should also implement NPC1 testing in all cases with unclear neuropsychiatric manifestations especially where a combination of hallucinations, epilepsy of any kind, facial dystonia and gait disorder is present.

A theoretical basis for using allele sharing distance to detect population stratification. *X. Gao* Miami Inst Human Genomics, Univ Miami Miller Sch Medicine, Miami, FL.

There is a long history of using allele sharing distance (ASD) and closely related metrics for population stratification analysis. However, the theory for this practical usage has not been reported. In this work, we describe the theoretical background for using ASD on single nucleotide polymorphism (SNP) genetic data for human population stratification analysis. In showing the proof, We lay the ground work for a general distance-based method for classifying subpopulations using SNPs and ASD.

High-resolution array-CGH Analyses of Common Hematological Malignancies Using Custom Designed Cancer-specific Oligonucleotide Arrays. *M. Li*[1, 2, 3, 4], *X. Hu*[1, 4], *D. Mercer*[1], *H. Safah*[3, 4] 1) Hayward Genetics Center; 2) Dept. of Pediatrics; 3) Tulane Cancer Center, Tulane Univ. Sch. Med; 4) Louisiana Cancer Research Consortium, New Orleans, LA.

Conventional cytogenetics and FISH have played crucial roles in the clinical diagnosis and treatment of hematological malignancies. However, the poor growth and/or poor morphology of malignant cells, along with limited FISH probe availability and resolution and sometimes conflicting results between cytogenetics and FISH, can make accurate clinical diagnosis difficult. We have designed a combined targeted-/whole-genome array system using the Agilent 4 x 44K format specific for cancer. 21,664 oligonucleotide probes were selected from the Agilent eArray system that target more than three hundred oncogenes, tumor-suppressor genes, and known cancer-associated chromosome regions. The average probe density was 71 probes per gene/region. Intervals between above genes or regions were filled relatively evenly with approximately 21,500 probes to cover the whole genome. To validate the array, we applied it to 20 cases of hematological malignancies, including AML, ALL, and multiple myeloma. Using the array, we not only confirmed most of the genetic aberrations identified by cytogenetics or FISH, but also clarified many controversial results between cytogenetics and FISH, and corrected misinterpreted FISH results. For example, a marker chromosome that was negative for the BCR/ABL1 fusion by FISH was proved to be a Philadelphia chromosome with a partial deletion; and a duplication of the p53 gene diagnosed by FISH was revised to be a rearrangement that had split the p53 gene probe. In addition, many small copy number alterations (CNAs), including intra-gene deletions and duplications were detected in patients with normal or abnormal karyotype. These CNAs appear to be significant in predicting patient response to therapeutic regimens and will cast new light on defining new genomic regions involved in disease pathogenesis. Our experience also highlights the importance of using combined targeted and whole-genome arrays in cancer diagnoses.

Association of GRIK1 gene in Schizophrenia: case-control and family-based studies. *Y. Hirata, N. King, JL. Kennedy* Neurogenetics section, the Centre for Addiction and Mental Health, Toronto , Ontario, Canada.

Purpose: One mechanism now emerging from genetic studies of schizophrenia worldwide is the glutamate system. Glutamate receptors mediate a vital part of neurotransmission in the mammalian central nervous system and play an important role in synaptic plasticity and the regulation of neurodevelopment. GRIK1 (GluR5 receptor) is expressed in the hippocampus and the Purkinje cells of the cerebellum. Thus our purpose was to examine genes that are implicated in the glutamate system. We have previously studied two of the glutamate system genes - GRIN1 and GRIN2B. In the current investigation, our focus was to analyze another important member of the glutamate system, GRIK1, in both case-control and family based samples for schizophrenia. Methods: We examined 11 SNPs across the GRIK1 gene in a sample of 182 case-control pairs and 104 small nuclear families. Genotyping was done using standard PCR methods. Statistical analyses were performed by using the Statistical package for the Social Sciences, version 10.0 (SPSS 2000) for the case-controls. For the family-based samples, the Family Based Association Tests (FBAT) were used. Results: In this study, we defined one block by Haploview 3.0 LD plot: 14Kb that included two SNPs. We did not observe any association with a specific allele, genotype or haplotype in our case-control samples. However, significant associations with alleles and genotypes were observed in our family-based samples, as follows for SNPs rs2832484 (p=0.020), rs2832489 (p=0.020), rs455892 (p=0.007), rs457474 (p=0.006), and rs460917 (p=0.006). For genotypes, we found some significant results in the family sample: rs2832484 (p=0.040), rs2832489 (p=0.040), rs455892 (p=0.039), rs457474 (p=0.030), rs460917 (p=0.030). Conclusion: Conclusions: We found an association between GRIK1 and schizophrenia in our family-based sample. On the other hand, our case-control study did not show any significant results. The family sample has a younger age of onset and stronger family history, and this may account for the difference in results between the two samples. Further work, at least in familial cases, is warranted.

1M SNP genome wide association for addiction: replicated results and comparisons of two analytic approaches.

G. Uhl[1], T. Drgon[1], C. Johnson[1], D. Walther[1], P.-W. Zhang[1], C.-Y. Li[1,2], J. Hess[1], Q.-R. Liu[1] 1) Dept Molec Neurobiology, NIH IRP (NIDA)Baltimore, MD; 2) Center for Bioinformatics, Coll Life Sci, Peking Univ, China 100871.

Addictions are substantially-heritable complex disorders whose molecular genetic bases have been only partially elucidated. We now report 1M SNP genome wide association (GWA) results for addiction vulnerability in each of two samples of ethnically-matched, well characterized substance dependent vs control subjects (n = 1620). We describe and compare converge then cluster and cluster then converge analytic approaches to assessing the convergence between the results obtained from the two samples, in conjunction with Monte Carlo and permutation statistics. The genes that we identify are expressed in brain regions that include hippocampus and amygdala much more than expected by chance. More are related to cell adhesion processes than expected by chance. Many of these genes help to establish and maintain neuronal connections. Others help to identify a number of readily-approachable candidate targets for antiaddiction pharmacotherapeutics. The methods that we describe to assess convergence between independent samples provide additional tools for assessment of convergent GWA results.

PTEN variant c.210-7~-3del5: low penetrance or modifying role in cancer predisposition? *J. Mester, P. Kessler, B. Leach, S. Gustafson, T. Sadler, F. Mularo, K. Waite, M. Orloff, C. Eng* Genomic Medicine Institute, Cleveland Clinic Foundation, Cleveland, OH.

PTEN encodes a phosphatase implicated in multiple cancer pathways. Germline deleterious mutations are present in 85% of individuals with Cowden syndrome (CS) and 65% with Bannayan-Riley-Ruvalcaba syndrome (BRRS). With 10 years of PTEN testing, some variations of uncertain significance (VUS) have been discovered. c.210-7~-3del5 appears pathogenic as it involves nucleotides close to the consensus intron 3 splice acceptor. After a total enrollment of 2,238 accruing by CS and CS/BRRS-like features over 2.5 years, 2 unrelated probands were found to have this VUS. A third proband presented to cancer genetics clinic with a referring diagnosis of VHL. The VUS was absent in 300 controls and 1,000 individuals with breast cancer only. The first proband, a 60-year-old female, had clinical features meeting CS diagnostic criteria including breast ductal carcinoma-in-situ (at age 59), thyroid cyst, 8 lipomas and macrocephaly. The second was a 4-year-old male with macrocephaly and developmental delay. The third proband was a 30-year-old female presenting with bilateral renal tumors and a pancreatic mass. She had normal intelligence and 2 Shagreen patches. Brain MRI revealed subtle tubers. We gave the clinical diagnosis of tuberous sclerosis (TSC) and found the TSC1 Q343X mutation. Her brother and father shared the VUS but neither had features of TSC or CS. PTEN RNA and protein studies in all 5 were normal: splice aberrations and upregulation of the AKT and MAPK pathways were not found. Literature review found the VUS in 3 TP53 mutation negative Li-Fraumeni syndrome (LFS)/LF-like patients. One had endometrial cancer and a family history of sarcoma, breast, lung, and colon cancer; 13 others studied were negative. In another series 2/26 had the VUS, one whose uncle had renal cell carcinoma. Both had normal PTEN mRNA studies. Taken together, these suggest that PTEN c.210-7~-3del5 may play a low penetrance or modifying role in broad cancer predisposition, notably of CS-related neoplasias. If this postulate is correct, then the VUS must carry out its role independent of known pathways.

Fanconi Anemia: Craniofacial Analysis Using 3-D Stereophotogrammetry. *R. T. McIntosh[1], D. L. Domingo[1], N. Tomona[1], R. Popat[1], S. Mitchell[1], N. Giri[2], B. P. Alter[2], T. C. Hart[1]* 1) NIDCR/NIH, Bethesda, MD; 2) CGB/DCEG/NCI/NIH, Bethesda, MD.

BACKGROUND: Fanconi anemia (FA) is a rare genetic chromosome instability disorder characterized by bone marrow failure, predisposition to malignancies & physical abnormalities (e.g., growth retardation). It consists of at least 13 complementation groups (FANCA,B,C,D1,D2,etc.) manifesting diverse clinical phenotypes. Craniofacial features (e.g., microcephaly, microphthalmia) remain poorly characterized. OBJECTIVE:To characterize facial features in FANCA patients using computerized surface models. METHODS: 16 Cauc FANCA patients (9males,7females,4-42yrs,median:18.7yrs) & 16 gender-, race- & age-matched healthy controls were imaged with stereophotogrammetry (3dMD System). The following age subsets were analyzed: <12yrs(n=4); 12-19yrs(n=5); & >20yrs(n=8). Using 14 facial landmarks, mean 3-D facial models were configured & analyzed with MorphostudioTM algorithms (finite-element analysis [FEA], function manager analysis [FMA]). RESULTS: All mean FA facial models appeared grossly smaller than the mean control models. Decreased facial volumes were most highly pronounced among FANCA patients age 12-19 compared to their controls. FEA measured the most significant reductions ($p<0.01$) in the following regions: glabella(15-32%), periorbital region(10-31%) & philtrum/perioral tissues(15-22%). FMA measured significantly shorter lower facial 3rd segments in the 12-19yr-old patients (mean:50.07mm vs. 58.24mm in controls)($p<0.05$). Among FA subjects >20 yrs, significant volume reductions ($p<0.01$) were observed in the philtrum/perioral(10-22%) & midface regions(9-12%). This oldest age group also had significantly narrower alar tissues (mean:30.44mm vs. 33.46mm in controls, $p<0.05$), although no significant vertical length reductions were detected. CONCLUSIONS: Non-radiation morphometrics quantified the abnormal facial gestalt of FANCA patients. Decreased surface volumes & linear segments reflect the growth retardation associated with this disorder. Quantification of facial compartments further defines previously reported FA facial features & may shed additional insight on this complex disease.

Power of model selection methods for high dimensional genome-wide association data. *Z. Wu, H. Zhao* Yale School of Medicine, Yale University, New Haven, CT.

Genome-wide association studies (GWAS) are characterized by the collection of hundreds of thousands of genetic markers and potential complex interactions among them to affect disease susceptibility. Although most commonly used, single marker based analysis is likely not optimal in the presence of multiple disease susceptibility genes and their interactions. Exhaustive search among models involving multiple markers is computationally intensive or even prohibitive, and it also suffers from an increased chance of finding false positive results due to the number of models (exponential in the number of SNPs jointly considered) explored. On the other hand, standard model selection methods, e.g. forward selection, may miss truly associated markers if disease susceptible genotypes/alleles only have small marginal effects yet large interaction effects. It is apparent that a delicate balance needs to be achieved between computational efficiency, statistical power, and low false positive rates, in the analysis and interpretation of GWAS data. Although highly important, this problem has only been partly explored to date by limited simulation studies. In this article, we derive analytical results for the statistical power of different marker selection methods, and address various factors that have impact on the choice of appropriate analysis methods.

ELAVL4: a depression-associated gene in Alzheimer and Parkinson disease. *M. Slifer[1], G. Beecham[1], G. Wang[1], E. Martin[1], J. Gilbert[1], J. Haines[2], J. Vance[1], M. Pericak-Vance[1]* 1) MIHG, Univ Miami, Miami, FL; 2) Vanderbilt, Center for Human Genetics Research, Nashville, TN.

To date, most genetic studies have focused on individual diseases. However, many complex diseases share features in common. Alzheimer (AD) and Parkinson (PD) disease are the two major neurodegenerative disorders of adults. The natural history of both diseases includes progressive cognitive and motor dysfunction. Molecularly, alpha-synuclein aggregates in the characteristic plaques of AD affected brains and Lewy bodies of PD affected brains. In addition, about half of those suffering from AD or PD develop depression. For this study, we explored the genetic basis of AD and PD with depression. As part of a larger case-control AD GWAS (n=988), using the Illumina 550K SNP marker set, we performed association testing on 207 AD cases with depression. All controls (n=496) are free of depression (i.e. euthymic) and cognitively intact by history and cognitive testing. AD with depression risk is associated with SNPs in ELAVL4 ($p<2.8x10-7$; odds ratio 1.8 (CI=1.5-2.3)) that exceed an FDR-BUM threshold for multiple testing. ELAVL4 is not associated with AD risk in a euthymic subset of AD (n=285; p=0.89). Interestingly, we had the initial report of an association for ELAVL4 with age-at-onset in PD. Subsequently, two other groups have found similar results (association with PD risk) in their independent PD datasets. Based on the AD results, we proceeded to test whether ELAVL4 is also acting through the depressed PD subset. When the PD dataset (n=754) was stratified by depression, the age-at-onset effect is only observed in the depressed subset of PD participants (n=198; p=0.003). The concurrence of results from two different common neurodegenerative diseases is remarkable, providing further evidence that common pathophysiological mechanisms may underlie both AD and PD and that ELAV4 is an important gene in both disease etiologies.

Potential mechanisms underlying tumorigenesis in *PALB2* mutation carriers with breast cancer. *N. Hamel[1], M. Couillard[1], A. D. Darnel[2], M. D. Tischkowitz[2], W. D. Foulkes[1,2]* 1) Dept Med Genetics, McGill Univ Health Ctr, Montreal, Canada; 2) Dept Med Genetics, Sir MB Davis Jewish General Hospital, Montreal, Canada.

PALB2 is a modest contributor to breast cancer susceptibility and germline mutations have been observed to segregate with breast cancer in several families. PALB2 is known to interact directly with BRCA2 and appears responsible for BRCA2 localization to the nucleus. While *PALB2*s contribution to breast cancer risk is clear, the mechanism by which heterozygous germline mutations lead to the development of breast tumors remains uncertain. Physical loss of the wild-type allele is a common mechanism by which heterozygous germline mutations in tumor suppressor genes become dominant in genomically unstable tumor cells. This phenomenon is frequently observed in *BRCA2*-related tumors. However, we did not observe similar loss of heterozygosity in breast tumors from 6 patients carrying one of 3 distinct *PALB2* truncating mutations, where mutant and wild-type *PALB2* alleles are all clearly retained. Since *PALB2* promoter methylation could be another means to suppress expression of the wild-type allele, we sequenced a 549bp CpG island including the transcription initiation site in tumors and matching blood samples from all patients, but failed to observe evidence of hypermethylation in tumors. These results suggest that wild-type PALB2 may still be expressed in *PALB2* tumors. Given the physical interaction between BRCA2 and PALB2, we speculated that simultaneous reduction in the levels of both proteins may conceivably result in an increased risk of tumor development; thus, we decided to also examine LOH and methylation status of *BRCA2* in our *PALB2* tumors. We found evidence of LOH at selected microsatellite markers flanking *BRCA2* in some tumors, but no consistent allelic loss across all tumors. Because *BRCA2* LOH in sporadic breast tumors is a frequent event, the significance of these observations is unclear. On the other hand, *BRCA2* promoter methylation has never before been observed in either sporadic or *BRCA*-related breast cancer, so any findings of hypermethylation here would be intriguing. *BRCA2* promoter methylation analysis is on-going.

Validation of an oligonucleotide microarray-based comparative genomic hybridization platform for clinical cytogenetic diagnosis. *S. Yu[1], D. Bittel[1], N. Kibiryeva[1], C. Saunders[1], M. Butler[2], D. Zwick[1], L. Cooley[1]* 1) Children's Mercy Hospital, Kansas City, MO; 2) 6410 Hillside St. Shawnee, KS 66218.

Comprehensive validation of any microarray platform is necessary to implement comparative genomic hybridization (aCGH) for clinical tests. Thirty two different specimens with previously identified chromosomal imbalances and thirteen blinded specimens were included in this study by using aCGH method with Agilents 244K oligo CGH microarray chips. All tests for this validation study were successful. The aCGH analysis was in agreement with all forty three samples with known chromosomal aberrations and the two remaining samples with reported normal results were confirmed as having no clinically relevant chromosomal aberrations. The chromosomal analysis results in three cases were improved by our aCGH-244K tests. In one case with 46,XX,der(4)ins(4;1)(35.2;q42.3q32.3), the inserted material was identified to be a 24.2 Mb DNA segment from chromosome 1p32.3p31.11 instead of 1q42.3q32.3. In one case with 46,XY,der(10)t(8;1)(q23;q26.3), our aCGH-244K results indicated a one-way translocation without involvement of chromosome 10q26.3. In one case with 46,XX,del(9)(q13q21.12), deletions at proximal ends of both arms of chromosome 9 were identified. Six cases with 22q11.2 deletion syndrome were found to have deleted regions ranging from 2.49 Mb to 2.56 Mb on one copy of chromosome 22q11.2. Four cases have common proximal breakpoints and five have common distal breakpoints. A candidate gene for autism (A2BP1) on chromosome 16p13.2 was deleted in a case which was confirmed as having a 199 Kb deletion involving the 5 end of this gene. A small number of copy number variations (CNVs) (~7/per sample) with sizes ranging from 400 bp to 1.6 Mb were revealed. With assistances from public and our own databases, as well as verification methods using quantitative real-time PCR, we see no difficulties in interpreting results from our aCGH-244K platform. These results demonstrate the utility of the array-CGH format as a powerful diagnostic tool for detection of genomic imbalances associated with genetic disorders.

Using Drosophila heart to uncover the genes contributing to Down syndrome congenital heart disease. *T. R. Grossman[1], A. Gamliel[2], R. J. Wessells[3], O. Taghli-Lamallem[4], K. Jepsen[2], J. R. Korenberg[5], M. G. Rosenfeld[2], R. Bodmer[4], E. Bier[1]* 1) Sec Cell & Dev Biol, Univ California, San Diego, La Jolla, CA; 2) HHMI, Dept Med, UCSD, La Jolla, CA; 3) Univ Michigan, Ann Arbor, MI; 4) Burnham Institute Med Res, La Jolla, CA; 5) Cedars-Sinai Medical Center, Los Angeles, CA.

Down syndrome (DS) is a major cause of congenital heart disease (CHD) and the most frequent known cause of atrioventricular septal defects. It has been suggested that DS-CHD is a multigenic disease that results from elevated expression of several genes from the DS-CHD candidate region, which include SH3BGR, DSCAM, WRB, Collagen VI A1 and A2, Collagen XVIII and HES1 genes. In attempt to identify which genes are responsible for DS-CHD we used the Drosophila heart as an in vivo assay system where we mis-expressed human and fly DS-CHD candidate genes using the UAS/GAL4 system and tested the effect on the heart physiology. In order to identify possible gene interactions we also mis-expressed all pairwise combinations. The effect on heart physiology was analyzed using sensitive heart function assays such as electrical pacing and high-speed video recordings from adult fly hearts. Our results show that mis-expression of certain DS-CHD candidate genes results in significant heart defects, including increased rate of pacing stress-induced heart failure. We found that out of all double gene combinations tested only two showed a synergistic aggravation of the heart phenotypes when simultaneously mis-expressed in the fly heart. In order to confirm our results in a mammalian system, we generated double transgenic mice with the two genes that we found to have the strongest interaction in the fly heart. Our preliminary data show that cardiac specific over-expression of two DS-CHD genes results in heart malformations. Taken together, our data suggest that two specific genes from the DS-CHD region on chromosome 21 may be responsible for the congenital heart defects in Down syndrome.

Scan of 640 SNPs of 43 candidate cleft lip or palate genes in the nonsyndromic cleft lip or palate patients of Lithuania. *V. Kucinskas, L. Ambrozaityte, A. Matuleviciene, E. Preiksaitiene* Human & Medical Gen, Vilnius Univ, Vilnius, Lithuania.

Orofacial clefts represent complex phenotype and reflect a breakage in the normal mechanisms during embryological development of the face. The incidence of cleft lip and/or cleft palate (CL/P) in the population of Lithuania is 1 in 544 newborns. As complex diseases may be caused by different causal mechanisms, many genes are considered as candidate loci for nonsyndromic CL/P responsible for this malformation. 104 triads of Lithuania of a child affected with nonsyndromic cleft lip and/or palate or cleft palate only and both his/her parents were included in this study. DNA microarray of 640 SNPs of 43 candidate cleft lip or palate genes was designed and produced by AsperBiotech, Estonia. The SNPs in the DNA microarray are distributed within and outside the genes. This experiment was carried out using arrayed primer extension - based genotyping technology (APEX-2). 20 SNPs were excluded from further analysis in the population of Lithuania having low call rate. Association statistics was performed by TDT/S-TDT, introduced by Spielman et al. 1993. This study showed statistically significant association of 18 SNPs (i.e. 2.9% of the investigated SNPs) to nonsyndromic cleft lip or palate. Six SNPs are at oddment distribution of the genes on the microarray - CDH1 gene (rs7188750 p=0.033), FOXE1 gene (rs973473 p=0.0338), LHX8 gene (rs17096272 p=0.003), MMP3 gene (rs629946 p=0.019), MMP9 gene (rs6032619 p=0.035), MSX2 gene (rs17063892 p=0.014). Three associated SNPs are of BMP2 gene - (rs17731603 p=0.014; rs6077060 p=0.039; rs235730 p=0.034). Most significant results involve/include FGF1, FGF2 and FGFR1 genes where nine SNPs in total show statistically significant results - (rs10064637 p=0.041; rs33995 p=0.004; rs10070885 p=0.004; rs249923 p=0.028; rs2034461 p=0.012; rs308434 p=0.012; rs308395 p=0.041; rs3804158 p=0.045; rs6987534 p=0.048). First results of our study suggest significant association of 18 allelic variants in ten out of 43 investigated genes that are possibly contributing to the aetiology and pathogenesis of nonsyndromic cleft lip or palate.

Cutis aplasia, facial dysmorphic features, congenital heart abnormalities and mental retardation in a child with a cryptic deletion in 19p13.3. *H. Al-Kateb[1], A. Hahn[2], J. M. Gastier-Foster[3,4], D. L. Thrush[3], L. Jeng[1], S. E. McCandless[2], C. A. Curtis[1]* 1) Univ Hosp Case Human Gen Lab, Case Western Reserve Univ, Cleveland, OH; 2) Univ Hosp Case Human Gen, Case Western Reserve Univ, Cleveland, OH; 3) Nationwide Children's Hospital, Columbus, OH; 4) The Ohio State University Columbus, OH.

Advances in technology have made it feasible to discover chromosomal microdeletions that are difficult to recognize by conventional chromosomal analysis. Here we report the use of chromosomal microarray, fluorescence in-situ hybridization (FISH), and molecular genetics techniques to delineate and characterize a de novo constitutional deletion within 19p13.3 in a girl with multiple congenital anomalies. Her phenotype includes cutis aplasia of scalp, structural heart abnormalities, macrocephaly, hypotonia, mild mental retardation, dysmorphic facies and conductive hearing loss. Review of the literature revealed a few case reports of larger deletions, most of which include the subtelomeric region, which appears to be intact in our patient. Initial microarray analysis (Signature Select 1.0) revealed a 6-BAC-clone deletion within 19p13.3 with an estimated deletion size of 1.612 Mb. FISH studies delineated the proximal deletion breakpoint to within BAC clone RP11-125C3 and the distal deletion breakpoint to a region within or near the proximal portion of BAC RP11-648B14. Using SNPs we determined that the deletion is of paternal origin and we refined the proximal deletion breakpoint by 50kb and confirmed the absence of BAC RP11-268O21, which is immediately proximal to RP11-648B14. Based on these findings, the deletion is estimated to be approximately 1.93 Mb, encompassing at least 72 genes. Two genes in the deleted region appear to be good candidates for the patients observed craniofacial and cardiac anomalies: guanine nucleotide binding protein (G protein), alpha 11 (Gq class)(GNA11) and Transducin-like Enhancer of Split 2 (E(sp1) homolog, Drosophila)(TLE2). Work to better define the breakpoints is ongoing, both to gain insight into the mechanism that led to this deletion and to correlate the genotype and phenotype of this patient.

Two independent alleles at 16p13 are associated with Addisons disease. *B. Skinningsrud[1,2], E. S. Husebye[3,4], S. H. Pearce[5], D. O. McDonald[5], K. Brandal[1], A. B. Wolff[3], K. Løvås[3,4], T. Egeland[1], D. E. Undlien[1,2]* 1) Department of medical genetics, Ullevål University Hospital, N-0407 Oslo, Oslo, Norway; 2) Institute of Medical Genetics, University of Oslo, N-0315 Oslo, Norway; 3) Section of Endocrinology, Institute of Medicine, University of Bergen, N-5021 Bergen; 4) Department of Medicine, Haukeland University Hospital, N-5021 Bergen, Norway; 5) Institute of Human Genetics, Newcastle University, Newcastle upon Tyne, NE1 3BZ, United Kingdom.

It is known that different autoimmune diseases often share the same susceptibility genes. In this study we aimed to investigate if loci found associated with common autoimmune diseases in recent genome wide association studies, also could be susceptibility loci for autoimmune Addisons disease. A total of 139 tagging SNPs in eleven candidate genes (IL2, IL21, IL2RA, CLEC2D, CD69, ERBB3, PTPN11, SH2B3, CLEC16A, CIITA and PTPN2) were genotyped in a case/control study design consisting of Norwegian Addisons disease patients (n = 332) and Norwegian healthy control individuals (n = 1029). Five SNPs were subsequently selected for analysis in a United Kingdom sample set consisting of Addison's disease patients (n =214) and controls (n = 190). Polymorphisms in CLEC16A and CIITA remained significantly associated with Addison's disease in the Norwegian sample set at the 0.05 level, even after correction for multiple testing. CLEC16A and CIITA are both located at 16p13, but linkage disequilibrium patterns and logistic regression analyses suggest that SNPs in these two genes are independently associated with Addison's disease. We were not able to confirm these associations in the UK material, however, this may well be due to limited sample size and lack of statistical power. In conclusion, two alleles at 16p13 are independently associated with risk of Addison's disease in the Norwegian population, suggesting this chromosomal region to harbour common autoimmunity gene(s), CLEC16A and CIITA being possible independent candidates.

Mapping of two new gene loci for Dandy-Walker Malformation. *M. Dasouki[1], D. Persons[2], G. Lushington[3]* 1) Dept of Pediatrics, Univ of Kansas Med Ctr, Kansas City, KS; 2) Dept of Pathology, Univ of Kansas Med Ctr, Kansas City, KS; 3) Molecular Graphics and Modeling Laboratory, Univ of Kansas, Lawrence, KS.

Dandy-Walker malformation (DWM) is a heterogeneous disorder which consists of cerebellar hypoplasia and and cystic dilation of the fourth ventricle often causing developmental delay, hypotonia, and ataxia. About 50% of affected patients have mental retardation and some have hydrocephalus. Both syndromic & non-syndromic, familial & isolated forms of DWM had been reported in the literature. Heterozygous deletion of the linked genes ZIC1 and ZIC4 was demonstrated to be involved in Dandy-Walker malformation in mice and humans. Here, we report on two unrelated children with DWM and chromosomal abnormalities that were identified prenatally. On prenatal ultrasound examination, patient 1 was found to have DWM. Pregnancy was terminated. No autopsy was done. An unbalanced karyotype [46,XX,del6p24.2,inv dup 8p11.2p21.3] was found in the product of conception. The mother had a normal phenotype with abnormal chromosomal complement [46,XX,t(3;6)(p26.2; p24.2),invdup 8p11.23p21.3]. At 3 years of age, patient 2 has coarse facies, speech delay, asymptomatic VSD, ASD & DWM. Blood chromosomal analysis showed an abnormal mosaic pattern: 46,XY,der(2)t(2;17)(q37.3;q25)(6)/46,XY(14). Array CGH & subtelomeric FISH probe analysis confirmed the partial trisomy for distal 17q25 and smaller partial monosomy for 2q37.3. In silico analysis of possible candidate genes in both regions revealed that NEDD9 is predicted to a fairly high probability to participate in short-hop protein-protein interaction networks with ZIC1 (via NEDD9-SMAD1-GLI3-ZIC1, NEDD9-SMAD2-GLI3-ZIC1, and NEDD9-SMAD3-GLI3-ZIC1), and is also predicted to share a common interaction partner (namely SMAD1) with the human ortholog ZNF423 whose mutation causes Dandy-Walker malformation in mice. These predictions, confirmed by experimental observations reported in KEGG, support the presence of a "DWM" locus that maps to distal 6p. The duplicated region of chromosome 17q contains several genes that encode multiple ciliary proteins that could be involved in DWM. Work is still ongoing to detect protein-protein interactions in this region.

***IGRP* is associated with type 2 diabetes and fasting plasma glucose levels in the Chinese population.** *C. Hu, C. Wang, R. Zhang, X. Ma, J. Xu, W. Jia, K. Xiang* Shanghai Diabetes Institute, Department of Endocrinology and Metabolism, Shanghai Jiao Tong University Affiliated Sixth Peoples Hospital, Shanghai, China.

Islet-specific glucose-6-phosphate catalytic subunit related protein (IGRP, also known as G6PC2) is a candidate for the low glucose-6-phosphate enzyme activity in islet and thus modulate glucose-stimulated insulin secretion. A recent genome-wide association study found the SNP rs560887 on *IGRP* was associated with fasting plasma glucose levels and beta-cell function in the general population. However, HapMap data showed that rs560887 was a rare variant in the Chinese population. Therefore, we used selected two SNPs (rs492594 and rs13387347) capturing all the common variants of this gene at r^2=0.8 based on HapMap CHB data and genotyped them in 3338 Shanghai Chinese using a SEQUENOM platform. The SNP rs13387347 was associated with type 2 diabetes in our population (OR 1.15 95% CI [1.05 - 1.27], *P* = 0.003, empirical *P* = 0.011 by 10,000 permutations). The rare allele C was significantly higher is the controls. By analyzing the quantitative traits in the controls, we found rs13387347 was associated with fasting plasma glucose concentration (*P*=0.0031). Our finding confirm the previous finding that *IGRP* was associated with glucose metabolism and reveal a novel associated SNP for type 2 diabetes susceptibility in the Chinese population.

Mutation negative mixed polyposis syndrome: polyp burden and cancer risks. *H. Leach, C. Eng* Genomic Medicine Inst, Cleveland Clinic, Cleveland, OH.

Literature review reveals only 2 large kindred with hereditary mixed polyposis syndrome (MPS). These authors characterize MPS by the presence of atypical juvenile polyps, adenomas, and colorectal cancer. 1 family was linked to BMPR1A, suggesting this to be a variant juvenile polyposis syndrome (JPS). Families with true MPS pose a challenge, as limited information is available about polyp burden and malignancy risk. We report on 64 unrelated patients with at least 5 gastrointestinal (GI) polyps of whic at least 1 is hyperplastic or hamartomatous. Patients were excluded from analysis if they met the diagnostic criteria for JPS, Peutz-Jeghers syndrome, PTEN-hamartoma tumor syndrome, or hyperplastic polyposis syndrome and were mutation negative for STK11, BMPR1A, SMAD4, PTEN, ENG, and MYH. Information was collected about polyp pathology, number, location, and age of onset and history of cancer. Mean number of colorectal polyps was 22/patient. Of the 64 patients, 26 underwent at least one upper endoscopy (EGD) and 7 (11%) had a mean of 8 polyps in the upper GI tract. Mean age of first polyp was 47 years, and ½ of the patients had 3 different types of polyps. 27 patients were diagnosed with cancer, of which 14 (22%) had colorectal cancer compared to 5% in the general population ($p < 0.0001$). The median age of colorectal cancer diagnosis was 56 years (mean 53 years, 99% CI 40-66), which is significantly younger than the general population median (71 yrs). 31 patients (48%) reported 1 first-degree relative (FDR) with polyps and 7 (11%) 1 second-degree relative (SDR) with polyps. 21 patients (33%) reported 1 FDR with colorectal cancer and 27 (42%) reported 1 affected SDR. The genetic etiology of polyposis in these patients is yet to be determined. However, given the breadth of polyp type and higher incidence of upper GI tract polyps and colon cancer, increased surveillance is indicated for patients and their family members. We recommend more frequent colonoscopies and at least a baseline EGD at the time of diagnosis. Patients should be counseled that the risk of colorectal cancer is increased, and family members should be advised that they are at increased risk for the same syndrome, potentially as high as 50%.

The Genetic and Rare Diseases Information Center (GARD) introduces a new online collection of information resources. *S. Von Schuch[1], M. Waite[1], J. Lewis[1], M. Della Rocca[1], D. Lea[2], H. Hyatt-Knorr[3]* 1) Lockheed Martin, Rockville, MD; 2) National Human Genome Research Insitute, Bethesda, MD; 3) Office of Rare Diseases, Bethesda, MD.

In 2001, the Office of Rare Diseases (ORD) and the National Human Genome Research Institute (NHGRI) at the National Institutes of Health (NIH) established the Genetic and Rare Diseases Information Center (GARD) to enhance the publics knowledge of genetic and rare disorders. Over the last 7 years, GARD has developed customized, comprehensive responses in English and Spanish to requests for information from patients, family members, health professionals and the general public. During this time, GARD has received close to 22,000 inquiries concerning more than 5,500 specific conditions. In 2008, GARD introduced a new online collection of information resources (http://rarediseases.info.nih.gov/GARD/) on the ORD Web site to provide the public with unlimited access to its extensive English-language information resources. Now when a person submits a question to GARD about a particular condition, the question is edited to ensure confidentiality and posted to the conditions Web page. Both the original inquirer and the general public will have access to the responses provided by experienced Information Specialists. A list of resources is also added to each conditions page to help users search for more information and find the answers to their questions. Resources are separated into different categories, including more information, support groups, research and clinical trials, services, and conferences. GARD Information Specialists remain available to assist users and answer any new questions. In 2009, GARD plans to expand its online collection to include Spanish-language information resources. The new GARD Web pages have averaged about 15,000 unique visitors since launching in February. The total number of visits has steadily increased over the past three months, with more than 45,000 visits during the month of April. As GARD receives more questions from the public on genetic and/or rare diseases, the collection of information and resources will continue to grow.

Investigation of GRIA3 Receptor Gene and Migraine Susceptibility. *T. Esposito[1], F. Fernandez[2], R. Lea[2,3], A. Aloia[1], R. Chimienti[1], A. Ciccodicola[1], G. Di Iorio[4], F. Gianfrancesco[1], L. R. Griffiths[2]* 1) Inst Genetics & Biophysics, CNR, Naples, Italy; 2) G.R. C., School of Med Sci, Griffith University, Gold Coast, Australia; 3) Inst of Environmental Sci and Research, Wellington, New Zealand; 4) Headache Service - Dep of Neurological Sci, II University of Naples, Italy.

Migraine is a debilitating neurological disorder characterised by recurrent attacks of severe headache, affecting 12% of Caucasian populations. Previously we reported the localisation of a migraine locus on chromosome Xq24-28. To define the associate region and to investigate for candidate genes we selected 19 candidate genes that, on the basis of their potential functional significance or physical position, may be involved in the pathophysiology of familial migraine. SNPs were selected in all described candidate genes and were genotyped in five Australian extended migraine pedigrees. Haplotype analysis and key recombination events showed two associated regions at Xq24 and the distal end of Xq28. This specific region contains a glutamate (GRIA3) receptor gene, which is a potential candidate gene for migraine susceptibility. We looked for functional SNPs, sequencing the exonic and regulatory region of GRIA3 gene in a cohort of Australian subject and we found an associated regulative variant -1722T/C. This variant was analysed in a large Australian population (275 unrelated Caucasian migraineurs versus 275 controls). Chi-square analysis showed significant differences in allele and genotype frequencies (P = 0.03). In order to replicate and validate these findings in a different population, we recruited an Italian cohort of migraine and control subject (150 migraineurs versus 150 healthy individuals). We genotyped the -1722T/C variant in the Italian panel and we found a significant association between this variant and migraine phenotype (p=0.003). Bioinformatics predictions showed that the C variant could affect putative binding sites for heat shock factor (HSF) altering the consensus sequence nGAAn, this change could alter the response of GRIA3 gene to stress condition. Further functional studies are currently underway.

PGD ON 2,438 EMBRYOS FROM 261 CYCLES DUE TO PARENTAL RECIPROCAL TRANSLOCATIONS, ROBERTSONIAN TRANSLOCATIONS OR PERICENTRIC INVERSIONS. *A. Benner, R. Pen, A. Kittai, W. G. Kearns* Shady Grove Center for Preimplantation Genetics, Rockville, MD.

Objective: Carrier couples with a structural chromosome rearrangement have an approximate 50% chance of producing genetically unbalanced gametes. Therefore, these couples are at high risk of producing genetically unbalanced embryos that can result in implantation failure, a miscarriage or a live birth with a genetic disorder. This study of 261 PGD cycles from referring clinics was performed to determine embryo structural chromosome balances due to parental rearrangements.

Materials and Methods: 171 patients underwent 261 *in vitro* fertilization (IVF) cycles for genetic imbalances due to parental structural chromosome rearrangements. Laser-assisted embryo biopsy was performed on 2,438 day-3 embryos. Multi-color fluorescence *in situ* hybridization using telomere and/or genomic loci DNA probes was used to determine embryonic genetic balances due to parental structural chromosome rearrangements. These included reciprocal translocations, Robertsonian translocations and pericentric inversions. Clinical pregnancy (CP) was defined by ultrasound identification of an intrauterine gestational sac and fetal heart beat.

Results: Thirty percent (79/261) of all cycles had no transfer because all tested embryos were either 1) genetically unbalanced or 2) genetically balanced but found to be morphologically abnormal in development and quality on the day of transfer. Seven percent (179/2,438) of embryos had no diagnosis because of poor embryo quality. The total CP rate for all structural chromosome aberrations was 23% (40/171). Breaking them down by the type of aberration shows a 23% (16/69) CP rate for reciprocal translocations, a 22% (18/83) CP rate for Robertsonian translocations and 32% (6/19) CP rate for pericentric inversions. The average biochemical pregnancy rate was 8.8% (15/171) per patient.

Conclusions: This data shows that PGD for structural chromosome abnormalities may increase the likelihood of a carrier couple of a structural chromosome rearrangement having a healthy baby.

Cystic Fibrosis prenatal screening of 103,600 individuals in an HMO: Molecular/clinical outcomes and a dramatic reduction in CF incidence. *D. Witt, C. Wold, P. Goonewardena, E. Louie, S. Rosenfeld* Genetics Dept, Kaiser Permanente, San Jose, CA.

We report on 8 years (11/99-12/07) of data from a cystic fibrosis (CF) prenatal screening program in an HMO. The program is unique because of its large size and integrated laboratory and clinical services that enable the analysis of comprehensive data and outcomes.

CF screening is offered in early pregnancy to couples in whom at least one partner is Caucasian. The pregnant woman is offered screening first, followed by her male partner if she is found to be a carrier. At-risk couples (both partners are carriers) are offered genetic counseling and prenatal diagnosis. Pregnancy termination is offered to couples with affected fetuses. The testing panel consists of 35 CFTR gene mutations.

Data are reported on 103,600 individuals with 3,514 carriers and 103 at-risk couples in 132 pregnancies. Specific CFTR gene mutations/frequencies will be reported. 87 pregnancies (66%) had prenatal diagnosis and 23 (26%) were affected. 17 of the 23 affected were predicted to result in severe CF of which 16 (94%) were terminated, and 6 of the 23 were predicted mild/asymptomatic of which 4 (67%) were terminated. 45 pregnancies (34%) did not undergo prenatal diagnosis but only 31 (23%) actively declined while the others were ineligible due to miscarriage or late presentation. The 45 untested pregnancies resulted in 11 affected babies of whom 6 were predicted to be severe and 5 predicted to be mild/asymptomatic. During the same period, 13 other babies with CF were born who were not identified prenatally either because of parental choice, test insensitivity and/or they did not fit ethnicity screening criteria. Overall, 52% (16/31) of pregnancies that would have led to the birth of a child predicted to have severe CF were terminated, thereby reducing the incidence of CF by approximately one-half.

The utility and benefits of CF screening as measured by personal choice and overall impact on disease incidence will be discussed.

Classification of genetic diseases for the clinician and the researcher: new tools developed by Orphanet. *S. Ayme, A. Rath, M. Georget, V. Lanneau, M. Hanauer* Orphanet, INSERM SC11, Paris, France.

Nomenclature of genetic diseases has evolved a lot during the past years, but no one knows how to establish the catalogue of human genetic diseases as a definition of what is a disease is lacking. OMIM is often cited as a catalogue of human genetic diseases when it is a catalogue of human genes. To overcome this difficulty, Orphanet (www.orpha.net) has established a database of phenotypes. The definition of what is a disease is the phenotype which has to be distinct for a clinician. Every phenotype is classified in the multiple possible classification systems to allow an understanding of its aetiology, of its physiopathology of or its range of expression. Every phenotype has a unique identifier which will remain stable and is linked to its parent disease and to its children diseases in the different classification systems. This includes all the published classifications and expert classifications established at Orphanet to meet the needs of the clinicians. They are based on the classical medical specialties and sub-specialties. Currently over 2,500 major phenotypes are classified and indexed. The users of the website can query by disease name at any level of precision, and by gene or by sign. They can visualise where the disease fits in the different classifications. This new service is expected to provide a bridge between clinicians and biologists for a mutual benefit. It is also expected to help make genetic diseases more visible in the health care information systems by providing a stable nomenclature of all existing phenotypes and to permit the interfacing of different databases. The data are available on request to all research and clinical groups which may need them.

Investigating the pathogenesis and therapy of Friedreich ataxia using mouse model cell lines. *C. Sandi, R. Mouro Pinto, S. Al-Mahdawi, M. A. Pook* CCCB/BICGP, Division of Biosciences, School of Health Sciences and Social Care, Brunel University, Middlesex, UB8 3PH, UK.

Friedreich ataxia (FRDA) is an autosomal recessive trinucleotide repeat disease caused by expanded GAA repeats in the first intron of the FXN gene. Normal individuals have 5 to 30 GAA repeat sequences, whereas affected individuals have 70 to 1600 repeats. The effect of the GAA expansion mutation is to reduce the expression of frataxin, a mitochondrial protein involved in iron-sulphur cluster biosynthesis. Evidence suggests that the GAA repeat expansion may adopt an abnormal triplex structure that interferes with FXN gene transcription. It has also been suggested that GAA repeats may produce a heterochromatin-mediated gene silencing effect. There is no effective therapy for FRDA. In order to gain further understanding of FRDA pathogenesis, the physiological function of frataxin and to develop an effective system for testing potential therapies, mouse models of FRDA are considered essential. We have recently generated a GAA repeat expansion mutation-based FRDA mouse model that exhibits GAA repeat instability, epigenetic changes and progressive mild pathology representative of FRDA. We now describe the establishment of fibroblast and neuronal stem cell lines from this FRDA mouse model. We find that mouse FRDA fibroblasts are significantly more sensitive to hydrogen peroxide-induced oxidative stress than control cells. We further show how these cultured cells are now being used to investigate epigenetic-based drug therapies for FRDA.

PREIMPLANATATION GENETIC SCREENING (PGS) FOR ANEUPLOIDY IN 93 COUPLES UNDERGOING DONOR EGG IN VITRO FERTILIZATION (IVF) CYCLES. *R. Pen[1], A. Benner[1], P. Kearns[1], A. Kittai[1], P. Browne[2], W. G. Kearns[1]* 1) Shady Grove Center for Preimplantation Genetics, Rockville, MD; 2) Shady Grove Fertility Reproductive Science Center, Rockville, MD.

Objective: To determine the prevalence of aneuploidy in couples undergoing donor egg IVF cycles, since PGS for aneuploidy is not a common recommendation for the donor egg patient group.

Materials and Methods: Ninety-three couples underwent donor egg IVF-PGS due to poor outcomes from prior fertility therapy. Laser-assisted embryo biopsy was performed on day-3 and PGS was done on 1,215 cleaving embryos from 93 initiated cycles. The mean donor age is 26.5 years (21- 31). Multi-color fluorescence *in situ* hybridization was used to determine aneuploidy for chromosomes 13, 14, 15, 16, 17, 18, 21, 22, X and Y. Hybridization, stringency washes and fluorescent microscopy were performed. Clinical outcomes (aneuploidy, embryo transfer, clinical pregnancies and delivery rates) of these cycles were determined. Clinical pregnancy was defined by ultrasound identification of an intrauterine gestational sac and fetal heart beat.

Results: All 93 women had an embryo transfer. Four percent (49/1,215) of the embryos were not diagnosed due to poor blastomere quality. Forty-eight percent (560/1,166) of the embryos were abnormal for at least one of the 10 chromosomes tested. Seventy-three percent (442/606) of the cytogenetically normal embryos developed into a blastocyst. The clinical pregnancy rate was 77% (72/93) per patient and 77% (72/93) per embryo transfer. There were no miscarriages, misdiagnoses, nor identified mosaic embryos.

Conclusions: This aneuploidy screening data from donor egg cycles provides insight into the presence of aneuploidy in a low risk population. Pregnancy rates were similar in these patients to those undergoing donor egg IVF without PGS. The miscarriage rate is 0% which may be due to the aneuploidy screening of the preimplantation embryos.

A Multicenter, Randomized, Dose Frequency Study of the Safety and Efficacy of Cerezyme Infusions Every 4 Weeks Versus Every 2 Weeks in the Maintenance Therapy of Patients with Type 1 Gaucher Disease. *P. Kishnani on behalf of the CZ-011-01 Study Investigators* Department of Pediatrics, Division of Medical Genetics, Duke University Medical Center, Durham, NC.

Introduction: Cerezyme (imiglucerase, Genzyme Corporation) is the current standard treatment for type 1 Gaucher disease (GD1). Most patients are infused every 2 weeks. A less frequent infusion schedule at the same total 4-week dose might be equally effective and more convenient. Objective: To compare the safety and efficacy of two dosing frequencies of Cerezyme for patients with GD1. Methods: A phase IV, multicenter, randomized 24-month trial enrolled 95 clinically stable adult GD1 patients (mean age 47 years; range 18-82 years) who had received Cerezyme 2 years. Patients were randomized to continue their total 4 week dose as 1 infusion every 2 weeks (Q2) or 1 infusion every 4 weeks (Q4). The primary analysis used a composite endpoint of relative change in hemoglobin level, platelet count, liver and spleen volumes, or progression of bone disease and bone crisis. A post-hoc analysis was performed based upon maintenance of therapeutic goals (Pastores et al. Sem Hematol 2004; 41(4 Suppl 5:4-14). Results: Mean 4-week doses (U/kg) of Cerezyme were 70.424.9 for Q2 (n=33) and 69.721.3 for Q4 (n=62). The primary analysis endpoint was maintained in 80.8% of Q2 and 63.2% of Q4 patients at 24 months. Per the post-hoc analysis, 100% of Q2 and 88.5% of Q4 patients maintained therapeutic goals at 24 months. No Cerezyme-related serious adverse events were reported. Conclusions: Infusing a total 4-week dose of imiglucerase every 4 weeks appears to be safe and well-tolerated in the majority of patients in this cohort and may be considered for some stable adult GD1 patients who have achieved therapeutic goals on Cerezyme therapy. The dosing frequency of Cerezyme, as with dose itself, must be individualized based upon physician judgment and patient needs as determined by regular and comprehensive monitoring. Long term follow-up of patients on a Q4 dosing regimen is necessary to determine other clinical outcomes, such as bone mineralization and marrow infiltration.

Identifying a risk factor for the development of gonadal tumors in patients with abnormal sexual development.

M. Beaulieu Bergeron[1,2,4], P. Brochu[1,2], M. Blumenkrantz[5], E. Lemyre[3,4], N. Lemieux[1,2,4] 1) Pathology Cell Biology, University of Montreal, Montreal, Canada; 2) Pathology, CHU Sainte-Justine, Montreal, Canada; 3) Pediatrics, CHU Sainte-Justine, Montreal, Canada; 4) Research Center, CHU Sainte-Justine, Montreal, Canada; 5) Pathology, McGill University Health Center, Montreal, Canada.

Patients with a disorder of sex development (DSD) and a Y chromosome are at risk of developing gonadal tumors, mainly gonadoblastomas (GBs) and dysgerminomas (DGs). These tumors would arise from germ cells that proliferate under the influence of TSPY (Yp11.2) in undifferentiated gonadal tissue (UGT) of dysgenetic gonads. On the contrary, gonadal streaks are thought to pose no threats of progressing into GBs as they do not contain germ cells. In order to verify this hypothesis, we retrospectively studied the gonads of 25 patients with a DSD and a Y chromosome who underwent prophylactic gonadectomy. The cohort included 10 females and 1 male with an isodicentric Y chromosome [idic(Y)], 1 female with a ring Y chromosome [r(Y)], 1 female with a Y chromosome derived from a translocation with an X chromosome [der(Y)t(X;Y)], 7 females and 1 male with a 46,XY constitution, and 2 males and 2 females with a 45,X/46,XY constitution. The region containing TSPY was still present in all patients. Histological review of gonads revealed the presence of UGT in 10 patients, streak gonads in 13 patients, and dysgenetic testes in 2 patients. 7 out of the 10 patients with UGT developed gonadal tumors: 4 females with an idic(Y) presented a GB, 2 XY females had bilateral DGs and/or GBs, and 1 45,X/46,XY female had a GB. As for the 3 patients with UGT and no tumors (a female with a der(Y)t(X;Y), a male with an idic(Y), and a 45,X/46,XY male), prophylactic gonadectomy at the very young age of 17, 13 and 19 months respectively might have prevented tumorigenesis. On the other hand, 1 female with an idic(Y) and no UGT developed a burned-out GB, although artefacts complicated the histological study. In conclusion, our retrospective study suggests that UGT is indeed at risk for developing GBs and/or DGs in individuals with a Y chromosome, including TSPY, and a DSD.

CHOP T/C and C/T Haplotypes Contribute to Early-Onset Type 2 Diabetes in Italians. *C. Gragnoli* Dept Endocrinology, Hershey Medical Ctr, Hershey, PA.

Type 2 diabetes (T2D) is characterized by impaired insulin secretion, insulin insensitivity and decreased beta-cell mass. Multiple genes contribute to T2D. The chromosome 12q13.1 region is in linkage to T2D in different populations, including our Italian dataset. CHOP is a candidate gene for the linkage, as it is located in the chromosome 12q13.1 region, and may contribute to T2D by increasing beta-cell apoptosis susceptibility and by impairing insulin sensitivity. Objective. Our goal was to identify any potential CHOP gene variants contributing to T2D in our Italian early-onset T2D families, which show linkage to the CHOP region. Material and Methods. We directly sequenced the CHOP gene in 28 Italian probands of the linked T2D families and in 115 control subjects. We performed genotype and haplotype association tests with T2D of the identified SNPs. We performed model-free and parametric association haplotype tests with T2D. Results. We identified three SNPs (5'UTR-c.279T>C, 5'UTR-c.120A>G and +nt30C>T (F10F) in CHOP. These SNPs are in complete linkage disequilibrium. The genotype association test showed an association trend with T2D of the TT (F10F) and AG (-c.120A>G). The haplotype association test provided significant results for the haplotypes T/C (frequency=0.33) and C/T (frequency=0.01) (at 5'UTR-c.279T>C and +nt30C>T, respectively) under non-parametric analysis (p-value=0.0000), recessive model (p-value=0.0000) and additive model (p-value=0.0014). Conclusions. Our data show that CHOP described haplotypes T/C and C/T, as an additive and as a homozygous variant, contribute significantly to T2D in our Italian early-onset group. We conclude that the CHOP T/C and C/T haplotype contributes to our T2D linkage signal on chromosome 12q13.1.

Clinical and Molecular Insights into Branchio-Oculo-Facial Syndrome (BOFS). *J. M. Milunsky[1,2], T. Maher[1], G. Zhao[1], D. Chitayat[3], M. Cunningham[4], W. Meschino[5], A. Megarbane[6], H. Stalker[7], R. Zori[7], A. Lin[8]* 1) Ctr. Human Genetics, BUSM, Boston, MA; 2) Dept. Pediatrics/Genetics and Genomics, BUSM, Boston, MA; 3) Dept. Pediatrics, Hosp. for Sick Children, Toronto, Canada; 4) Dept. Pediatrics, University of Washington, Seattle, Washington; 5) Dept. Pediatrics, University of Toronto, Toronto, Canada; 6) Unit of Medical Genetics, University Saint-Joseph, Lebanon; 7) Div. of Genetics, University of Florida, Gainesville, FL; 8) Genetics Unit, MGH, Boston, MA.

Branchio-Oculo-Facial Syndrome (BOFS) is a rare autosomal dominant cleft palate-craniofacial disorder with variable expression. Major features include cutaneous and ocular anomalies, typical facies, and less commonly, renal and ectodermal anomalies. We recently determined that mutations involving *TFAP2A* result in BOFS (Milunsky et al, 2008). We have now studied a total of 10 families (14 affected individuals) that meet the cardinal diagnostic criteria. The 3.2Mb deletion including the *TFAP2A* gene detected in family 1(affected son/mother)has also been found in the maternal grandmother who has a branchial cleft cyst and hearing loss. Members of this family were the only BOFS patients without typical CL/P. 9/10 families had missense mutations detected in the *TFAP2A* gene in the highly conserved exons 4 and 5 (basic region of the DNA binding domain). The missense mutations appear to cluster in an apparent hotspot region between amino acids 254 and 256 (6/9 patients). Mutations of amino acid 254 occurred in 4 unrelated families (R254G/R254W). In one of these families, the father (white forelock, pre-auricular sinus and supernumerary nipple) appears to be a mosaic (R254G) in blood. Thus far, genetic heterogeneity has not been observed. The clustering of mutations supports a tiered molecular approach to the analysis starting with sequencing of exons 4 and 5 and, if negative, full sequencing of the gene followed by deletion analysis if unrevealing. There was some clinical variability with the deletion family, with little variability between patients having missense mutations. More patients need to be studied to better appreciate if there are mutation-specific genotype phenotype correlations.

Increased copy number of genes encoding the sister chromatid cohesion complex conveys a syndrome distinct from Cornelia de Lange. *J. R. Lupski[1,9,10,11], F. Zhang[1], E. Brundage[1], A. Scheuerle[2], B. Lanpher[3], R. P. Erickson[4], Z. Powis[4], H. B. Robinson[5], P. L. Trapane[6], D. Stachiw-Hietpas[7], K. M. Keppler-Noreuil[8], S. R. Lalani[1,11], T. Sahoo[1,11], A. C. Chinault[1], A. Patel[1,11], S. W. Cheung[1,11], J. Yan[1]* 1) Mol & Human Gen, Baylor Col of Med; 2) Tesserae Gen, TX; 3) Dept of Pedi, Vanderbilt U, TN; 4) Dept of Pedi, U of Arizona, AZ; 5) Dept of Path, Akron Children's Hospital, OH; 6) Med Col of Wisconsin; 7) Children's Hospital of Wisconsin, WI; 8) Dept Pedi/Med Gen, U of Iowa, IA; 9) Dept. of Pedi, Baylor Col of Med; 10) Texas Children's Hospital; 11) Med Genet Lab, TX.

Cornelia de Lange Syndrome (CdLS) is a multisystem congenital anomaly disorder. Heterozygous mutations in three genes (*NIPBL*, *SMC3* and *SMC1A*), encoding components of the sister chromatid cohesion apparatus, are responsible for ~50-60% of CdLS cases. Recent studies have revealed a high degree of genomic rearrangements (e.g. deletions and duplications) in the human genome, which result in gene copy number variations (CNV). CNVs have been associated with a wide range of both Mendelian and complex traits including disease phenotypes such as Charcot-Marie-Tooth type 1A, Pelizaeus-Merzbacher, Parkinson, Alzheimer, autism and schizophrenia. Increased versus decreased copy number of the same gene can potentially cause either similar or different clinical features. We identified duplications on chromosomes 5 or X using genome wide array Comparative Genomic Hybridization (aCGH). The duplicated regions contain either the *NIPBL* or the *SMC1A* genes. The patients share some common features including mental retardation, developmental delay, sleep abnormalities, and crainofacial and limb defects. The systems affected are the same as in CdLS, but clinical manifestations are distinct from CdLS. Our results confirm the notion that CNV of genes can be a common mechanism for human genetic diseases. Defining the clinical consequences for a specific gene dosage alteration represents a new reverse genomics trend in medical genetics that is reciprocal to the traditional approach of delineation of the common clinical phenotype preceding the discovery of the genetic etiology.

Optimizing approximate Bayesian computation methods for sequence-based studies of population history. *R. L. Raaum[1,2], C. J. Mulligan[3]* 1) Anthropology, Lehman College & the Graduate Center, City University of New York, New York, NY; 2) New York Consortium in Evolutionary Primatology; 3) Anthropology, University of Florida, Gainesville, FL.

Many Bayesian methods used in population genetic studies are computationally intensive and may not be practically applied to all questions of interest. One of the most promising new methods being developed is approximate Bayesian computation (ABC). In this method summary statistics from the observed data are compared to those calculated from simulated data to estimate parameters of interest. However, ABC is a relatively new approach and few of the analytic choices for this method have been thoroughly evaluated. Here we present the results of our analysis of some of these choices. First, we determined when the computationally efficient infinite sites model may be used in the simulations instead of a more complex model (e.g. HKY). We modeled four types of commonly studied human sequence data: mitochondrial hypervariable, mitochondrial coding, autosomal non-coding, and Y non-coding. We found that parameter estimates based on HKY simulations were significantly better than the infinite sites based estimates for both mitochondrial sequence types, but that the estimates did not differ significantly for the two nuclear sequence types. Second, we determined if using more summary statistics improves the estimate by adding more information or worsens the estimate by introducing noise. For this, we calculated parameter estimates using 2, 4, or 6 statistics, and found no statistically significant difference among any of these estimates. Third, many studies have used nucleotide diversity (pi) and number of segregating sites (ss), but these statistics have not been demonstrated to be more accurate or precise than other potential statistics. To determine the best set of statistics, we compared the accuracy and precision of parameter estimates based upon all possible pairs of a selection of 11 statistics. We found that pi and ss is not a universally optimal selection for the population models we evaluated, and that no single pair of statistics is universally optimal for commonly estimated parameters.

Mutations in the Type I Collagen C-propeptide Cleavage Site Cause a Distinct Phenotype Based on Slower Procollagen Processing. *A. M. Barnes[1], K. Lindahl[2], M. Whyte[3], T. Hefferan[4], C.-J. Rubin[2], A. Kindmark[2], O. Ljunggren[2], J. C. Marini[1]* 1) BEMB, NICHD/NIH, Bethesda, MD; 2) Dept of Endocrinology, Medical Sciences, Uppsala University, Uppsala, Sweden; 3) Shriner's Hospital for Children, St. Louis, MO; 4) Dept of Orthopedics, Mayo Clinic, Rochester, MN.

Osteogenesis imperfecta (OI), or brittle bone disease, is often caused by mutations in the type I collagen genes; over 800 have been described. Mutations in type I procollagen C-propeptide cleavage site are of particular interest because they disrupt a unique processing step. We identified two children with mild OI who had cleavage site mutations in *COL1A1* (P1: 1(I)Asp1041Asn) or *COL1A2* (P2: 2(I)Ala1029Thr). P1 had a normal *LRP5* sequence. P1 DEXA Z-score and pQCT vBMD were +3, contrasting with radiographs demonstrating osteopenia and os-in-os vertebrae, and histomorphometry revealing increased bone remodeling, without a mineralization defect or signs of osteosclerosis. P2 had a DEXA z-score of 0, gracile long bones with radiographic osteopenia, and decreased BV/TV and increased BFR without a mineralization defect on histomorphometry. Both P1 and P2 are at the 75th percentile for height. The dermal fibrils of P1 had a normal diameter with a smooth surface, while P2 had small fibrils including some with blebs. Steady-state collagen electrophoresis showed slight backstreaking of 1(I) and 2(I) in cell layers of both probands. The baseline of P1 chains was delayed, while those of P2 migrated normally. Chain incorporation was normal in P1 and slightly delayed in P2. Pericellular processing of P1 was delayed, with increases in both pC1 and pro2, while P2 had increased pC2 and pro2 and normal processing kinetics. These mutations seem to define a novel phenotype within type I collagen defects. In combination with a recently reported adult with pro1(I)Ala1040Thr substitution (Int Conn Tis 82S1: CC01), our cases suggest that defects in pro1(I) processing can lead to high BMD in childhood, with radiographic signs of osteopetrosis occurring subsequently. Pro-1(I) cleavage appears crucial to C-propeptide processing, while defective pro-2(I) specific or non-specific cleavage occurs after 1(I) processing.

New candidate genes for osteoporosis identified by linkage and linkage disequilibrium analysis of QTL on chromosome 1p36. *H. Zhang[1], K. Sol-Church[2], H. Rydbeck[1], D. Stabley[2], L. D. Spotila[3], M. Devoto[1,4,5]* 1) The Childrens Hospital of Philadelphia, Philadelphia, PA; 2) Nemours Childrens Clinic, Wilmington, DE; 3) ScienceScribe, Haddonfield, NJ; 4) Dept. of Pediatrics and Dept. of Biostatistics and Epidemiology, University of Pennsylvania School of Medicine, Philadelphia, PA; 5) Department of Experimental Medicine, University La Sapienza, Rome, Italy.

Low BMD is one of the major risk factors for osteoporosis. Following a whole genome scan in a sample of Caucasian families recruited through probands with low BMD, a region on 1p36 near marker D1S214 received support as a candidate quantitative trait locus (QTL) for BMD from both linkage (maximum lod-score = 2.87) and linkage disequilibrium analysis ($P < 0.01$). In an attempt to better characterize the genetic risk factors for low BMD located in this genomic region, we have now genotyped the same group of families for 1095 single nucleotide polymorphisms (SNPs) located across 11 Mb on chromosome 1p36 at an average spacing of 10 Kb. Linkage and linkage disequilibrium analyses have been performed by means of the variance component approach. Multivariate variance component linkage analysis indicated two QTLs for femoral neck BMD, lumbar spine BMD and trochanter BMD simultaneously on chromosome 1p36, with maximum lod-scores of 4.37 at 12 cM (~3.7 Mb) and 3.59 at 22 cM (~8.2 Mb). Linkage disequilibrium analysis identified several SNPs potentially associated with BMD, including the RERE gene intragenic SNP rs11121179 ($p = 0.000005$ for lumbar spine BMD) and the intergenic SNP rs579992 ($p = 0.00004$ for femoral neck BMD). Other candidate genes in this region include G1P2, SSU72 and CCDC27 ($p < 0.001$ for association with at least one of the BMD traits). This study supports the presence in 1p36 of QTLs that affect BMD at multiple skeletal sites and suggests several new candidate genes for further analyses. Replication of our results in other independent cohorts is warranted.

Haploinsufficiency of *Rai1* causes features reminiscent of metabolic syndrome. *J. Yan[1], W. Bi[1], P. K. Saha[2], L. Chan[2], J. R. Lupski[1,3,4]* 1) Dept Molecular & Human Gen, Baylor Col Medicine; 2) Department of Molecular and Cellular Biology; 3) Department of Pediatrics, Baylor College of Medicine; 4) Texas Children's Hospital, Houston, TX.

Heterozygous mutations in the *RAI1* gene are responsible for the majority of clinical features in the Smith-Magenis syndrome (SMS), a multiple congenital anomaly disorder. Both *Df(11)17*/+ mice, with a heterozygous 2 Mb deletion in the mouse chromosome 11 region syntenic to the SMS critical region, and *Rai1*+/- mice manifest abnormalities recapitulating some SMS clinical phenotypes, including craniofacial defects and obesity. The craniofacial abnormalities and obesity are normalized in *Dp(11)17*/*Rai1*+/- animals, trisomic for ~20 genes but disomic (N=2) for *Rai1*, demonstrating these traits or endophenotypes are caused by *Rai1* gene dosage. Further characterization of the obesity phenotype demonstrated that *Rai1*+/- mice had increased food intake and stored more fat in individual adipose cells. The blood cholesterol levels were increased, which has also been observed in individuals with SMS. Glucose tolerance test indicated insulin insensitivity. The leptin levels were elevated in the *Rai1*+/- mice. These features are reminiscent of metabolic syndrome. Our mouse model thus provides an opportunity to study not only the molecular mechanisms for multiple features in SMS but also common traits such as obesity.

Assessment of Carboxypeptidase M (CPM) Amplification: A surrogate marker for MDM2 amplification in the discrimination of well-differentiated liposarcomas from lipomas. *M. R. Johnson, A. R. Seys, C. W. Roth, A. A. King, R. L. Hulshizer, X. Wang, R. V. Lloyd, A. M. Oliveira* Mayo Clinic, Rochester, MN.

Background. Discrimination of well-differentiated liposarcoma/atypical lipomatous tumor (WDL/ALT) from lipoma can be diagnostically challenging. However, cytogenetic identification of ring and giant rod chromosomes strongly support the diagnosis of WDL/ALT. These abnormal chromosomes are mainly composed of sequences derived from bands 12q13-15, which contain several amplified genes including MDM2, CPM, CDK4, TSPAN31, and others. MDM2 is amplified in 99% of WDLs, and up to 30% of other sarcomas. CPM encodes for carboxypeptidase M, a membrane-bound peptidase that is located 11 kb upstream from MDM2. A comparative assessment of MDM2 and CPM was conducted on a series of lipomatous tumors to discriminate WDL/ATL from lipomas. Design. Seventeen WDL/ALT, 22 ordinary lipomas, and 16 other mesenchymal tumors were evaluated by MDM2 and CPM amplification using FISH on 4 m paraffin-embedded tissue sections. All experiments were performed by co-hybridizing MDM2 or CPM (custom designed probes) with a commercially available centromere 12 specific probe (CEP12, Vysis). Two hundred cells/tumor were evaluated by 2 technologists without prior knowledge of the diagnosis. Results. All WDL/ATL showed amplification of both MDM2 and CPM (usually 20 copies/cell). All lipomas and the 16 other mesenchymal tumors were negative for MDM2 or CPM amplification. Conclusions. CPM was co-amplified with MDM2 amplification in 100% of WDL/ALT but in none of the other tumors evaluated; including the 22 ordinary lipomas. Therefore, FISH for CPM amplification can be used as an alternative diagnostic tool for the diagnosis of lipomatous neoplasms.

Initial experiences with the Affymetrix 6.0 SNP Array. *M. de Andrade[1], E. Atkinson[1], W. Bamlet[1], M. Matsumoto[1], S. Maharjan[1], S. Kardia[2]* 1) Div Biostatistics, Mayo Clinic, Rochester, MN; 2) Epidemiology, University of Michigan, Ann Arbor, MI.

In an era of genome-wide association analyses, researchers are facing the challenge not only of analyzing a large volume of data but also of processing the genotype data and creating appropriate workflows. We present our experiences in working with the Affymetrix 6.0 Genome-Wide Human SNP Array including the workflows that we have created. In particular, we focus on the issues prior to genotype extraction, assessment of genotype accuracy, and automation of the workflows recognizing that the Affy 6.0 data will eventually be used for analysis using a wide range of study designs. We will share our experience working with Birdseed1 and 2 using individual plate and all plates to generate genotype call, examining replicate samples and the challenges with quality control measures in sibships. We will present our workflow and initial results using 900 samples of hypertensive sibships from Rochester, MN.

Deletion of 6q25-qter: Candidate region for brain and urinary system development genes? *N. Qin[1,2], E. Mahmoud[3], A. Hajianpour[4], M. Kanzawa[1], B. Huang[1,2]* 1) Dept Cytogenetics, Genzyme Genetics, Orange, CA; 2) Division of Human Genetics UCI; 3) Division of Neonatal-Perinatal Medicine UCI Medical Center; 4) Dept Cytogenetics, Genzyme Genetics, Monrovia, CA.

We report here a male infant with a terminal deletion of 6q25.3-qter and having apparent brain and urinary tract system malformations more severe than those described in most published cases. The proband was born at 39 weeks gestational age by C-section to a 19 year-old, gravida 2, para 2, Hispanic mother and a 28 year-old father. The APGAR Scores were 7 and 7 at one and five minutes of life. Pregnancy was complicated by the finding of hydrocephalus, cleft lip and palate at 24 weeks of gestational age. Chromosome analysis on amniotic fluid revealed a normal male karyotype at 450 band level (done at another laboratory). Parents continued pregnancy. At birth, the infant was observed to have hydrocephalus with open fontanels, hypotelorism, midline cleft lip and palate, moderate abdominal distension, bilateral clenched fists and rocker bottom feet. Cranial ultrasound reported severe hydrocephalus, minimal brain substance (consistent with holoprosencephaly). The Abdominal X-ray demonstrated a large fluid-filled intra-abdominal mass. The Abdominal ultrasound showed a small liver, normal right kidney, however, failed to visualize the left kidney and the bladder. Postnatal cytogenetic analysis on peripheral blood showed a subtle terminal deletion of the long arm of one chromosome 6 at 550 band level, which was confirmed by FISH studies. Patient died at about 17 hours of life. The presence of a subtle terminal deletion of the long arm of chromosome 6 with multiple congenital abnormalities may suggest high gene density in chromosome band 6q25-ter region. These genes may be sensitive to haploinsufficency, as evident from the infants severe abnormal phenotype, especially those major abnormalities detected in his brain and urinary tract system. In conclusion, a 6q25-qter segment may serve as a candidate region for genes involving in brain and urinary tract system development.

Two cases of Maple Syrup Urine Disease patients detected by Neonatal Screening Test with four novel mutations of BCKDHA in Korea. *I. Choi[1], J. Ko[2], G. Kim[1], J. Lee[1], C. Cheon[2], H. Yoo[1,2]* 1) Medical Genetics, Asan Medical Center, Seoul, Korea; 2) Dept. of Pediatrics, Asan Medical Center, Ulsan College of Medicine, Seoul, Korea.

Maple syrup urine disease (MSUD) is an autosomal recessive disorder involving the metabolism of the branched chain amino acids (BCAA) such as leucine, isoleucine and valine. This disorder results from a defect in branched chain -ketoacid dehydrogenase (BCKDH) complex. Impaired activity of BCKDH complex causes accumulation of branched-chain L-amino and 2-oxo acid, leading to neurotoxic damage to developing brain. Disease-causing mutations have been identified in the BCKDHA, BCKDHB or DBT genes encoding for the E1, E1; and E2 subunits of the BCKDH complex. MSUD presents with varying clinical features. Their genotype is also heterogeneous. Severity of the disease, ranging from classical to mild variant type, is commonly classified on the basis of various characteristics such as onset of symptom, responsiveness to thiamine therapy, and residual enzyme activity. We report two unrelated Korean MSUD patients, 10 and 17 day-old girls, who were detected by tandem mass spectrometry and confirmed by BCKDHA and BCKDHB gene analysis. They did not show any sign of neurological deterioration except increasing level of serum leucine (13.6 and 34.6 mg/dL) on repeated tandem mass screening test. BCAA-restricted diet and thiamine supplementation were prescribed. Leucine (808 and 4109 mol/L) and alloisoleucine levels were elevated on serum aminoacid analysis by HPLC, and -ketoacid (2-ketoisocaproic acid, 2-ketoglutaric acid) was detected in their urine. We performed BCKDHA and BCKDHB gene analysis using patients leukocyte, and they were confirmed as compound heterozygotes with four novel sequence changes (p.Arg279His, p.Pro324_Lys325dup, p.Ser46GlnfsX17, p.Thr211Met) in BCKDHA gene. They are 8 and 11 months old now, and develop normally without neurological complication. MSUD is a fetal disease, however better outcomes are expected if early diagnosis and prompt management are provided.

Copy number variations in patients with overgrowth syndromes detected by array-CGH. *V. Malan[1,2], S. Chevallier[1], C. Coubes[3], D. Lacombe[4], L. Pasquier[5], J. Soulier[6], N. Morichon-Delvallez[1,2], M. Vekemans[1,2], A. Munnich[1,2], V. Cormier-Daire[1,2], L. Colleaux[1,2]* 1) Département de Génétique et INSERM U781, Hôpital Necker Enfants Malades, Paris, France; 2) Université Paris Descartes, Paris, France; 3) Service de Génétique Médicale, CHU Saint- Eloi, Montpellier, France; 4) Service de Génétique Médicale, CHU Pellegrin, Bordeaux, France; 5) Service de Génétique Médicale, Hôpital Sud, Rennes, France; 6) Laboratoire d'Hématologie, Hôpital Saint- Louis, France.

Overgrowth syndromes are a heterogeneous group of conditions including endocrine hormone disorders, several genetic syndromes and many situations with thus far unexplained mechanisms.Interestingly, chromosomal anomalies have been identified in patients with overgrowth such as dup(4)(p16.3),dup(15)(q26qter)and del(9)(q22.3q22.33).Thus,we hypothesized that the sensitivity of array-CGH could improve the genetic diagnosis of overgrowth conditions. Eighty six patients with unexplained overgrowth syndrome were analyzed using a 1 Mb resolution array-CGH. Patients were classified into two groups: group I (40 cases) includes patients with a clinically known syndrome (i.e Sotos syndrome) whereas group II (46 cases) includes patients with unclassified overgrowth syndrome.We detected 10 possibly pathogenic imbalances in 9 patients (11.6%): two belong to group I while 7 belong to group II. Four imbalances correspond to deletions and 6 to duplications.No recurrent abnormality was identified.FISH analyses confirmed the chromosomal abnormalities in 7 cases while the remaining cases are still under investigation.Firstly, these results demonstrate that array-CGH is able to provide a high diagnostic yield in patients with overgrowth syndrome. Secondly, while chromosomal deletions are most often associated with growth retardation, we found that the majority of the imbalances detected in our patients are duplications.Thirdly, careful re-examination of patients may allow the delineation of novel clinically recognizable overgrowth syndromes. Finally, besides their importance for diagnosis and genetic counseling, these data may pave the way to the search of genes involved in the pathogenesis of overgrowth.

Pathogenic Copy Number Variations (CNVs) in patients with borderline intellectual functioning. *C. Romano, S. Reitano, D. Greco, E. Avola, P. Failla, G. Belfiore, S. Buono, M. Elia, O. Galesi, L. Castiglia, M. Fichera* IRCCS Associazione Oasi Maria Santissima, Troina, Italy.

Array CGH in patients with mental retardation and normal karyotype, shows causative CNVs in a percentage of 5-25% depending on techniques used and selection of patients. We applied this new technique in 22 patients classified as Borderline Intellectual Functioning (BIF) with a global intelligence quotient (IQ) in the range 71-84, according to DSM-IV-TR criteria, and we found 5 patients carrying a clinically relevant CNV (22.7%). The clinical checklist published by De Vries et al. (2001) was administered for each patient and showed that 3/5 (60%) carriers of chromosomal rearrangements and 11/17 (64.7%) patients negative to array-CGH analysis had a score of 3 or above. Candidates to array-CGH analysis are frequently patients with mental retardation and a Chromosomal phenotype, but our results suggest that this selection may not be the best clue for the diagnosis of chromosomal aberrations.

Novel polymorphisms in PPARG and ADIPOQ genes increase type 2 diabetes risk in Asian Indian Sikhs: Evidence of gene-gene interaction. *D. K. Sanghera[1], Y. F. Demirci[2], L. Ortega[1], L. Been[1], S. K. Ralhan[3], G. S. Wander[3], N. K. Mehra[4], J. R. Singh[5], J. J. Mulvihill[1], M. I. Kamboh[2]* 1) Dept Pediatrics, Univ Oklahoma HSC, Oklahoma, OK; 2) Department of Human Genetics, Univ of Pittsburgh, PA; 3) Hero DMC Heart Institute, Ludhiana, India; 4) All India Institute of Medical Sciences and Research, New Delhi, India; 5) Guru Nanak Dev Univ, Amritsar, India.

Asian Indians, 25% of world populations comprise highest number of diabetics of the world, reasons underlying the high prevalence are not understood. Studies point to strong genetic predisposition in response to certain environmental factors. In this study, we have examined the roles of peroxisome proliferator activated-receptor gamma (*PPARG*) and adiponectin (*ADIPOQ*) genes for affecting type 2 diabetes (T2D) risk in Asian Indian Sikhs. We genotyped 15 tagSNPs in *PPARG* and 5 tagSNPs in *ADIPOQ* in 562 T2D cases and 540 controls and examined their association with T2D. Three SNPs in the *PPARG* gene were significantly associated with T2D under recessive (rs1175073, p=0.021; rs1801282/Pro12Ala, p=0.007) and dominant (rs3892175, p=0.008) models after adjusting for the effects of age and sex. Also, two SNPs from *ADIPOQ* gene (rs182052, p=0.036 and rs7649121, p=0.034) were significantly associated with T2D under dominant model. In two-point haplotype analysis, GC haplotype of rs3892175 and rs1801282 in *PPARG* revealed significant association with T2D (^{2}=10.3; p=0.001; permutations p=0.002). Similarly, GA haplotype of rs182052 and rs7649121 in *ADIPOQ* showed significant association with T2D (^{2}=6.60; p=0.010; permutations p=0.03). In gene-gene interaction, rs3892175 and rs1801282 from *PPARG* revealed a strong interaction with rs7649121 from *ADIPOQ* in response to increasing the risk to T2D (p=1.08X10^{-3}). To further confirm this, we tested the association of T2D among risk allele carriers. The risk alleles of three interacting SNPs; rs3892175, rs1801282, and rs7649121 showed the strongest association with T2D (p=3.72X10^{-4}) compared to all other SNPs and alleles tested. Our new findings strongly suggest that the genetic variation in *PPARG* and *ADIPOQ* loci could be a major risk factor for the development of T2D in Indian Sikhs.

The recurrent mutation p.R674Q in the perinatal myosin heavy chain gene (MYH8) is associated with trismus-pseudocampodactily (TPS) in two brothers coming from Calabria, a region of Southern Italy. *G. Bonapace[1], F. Ceravolo[1], M. G. Pascale[1], S. Sestito[1], R. Apa[1], M. T. Moricca[1], P. Strisciuglio[2], D. Concolino[1]* 1) Dept of Pediatrics, Univ Magna Graecia, Catanzaro, Italy; 2) Dept of Pediatrics, Univ "Magna Graecia", Catanzaro, Italy; Present address: Dept of Pediatrics Univ Federico II, Naples, Italy.

Introduction:TPS is a rare autosomal dominant distal arthrogryposis characterized by an inability to open the mouth fully (trismus) and an unusual camptodactyly of the fingers that is apparent only upon dorsiflexion of the wrist . TPS is also known as Dutch-Kentucky syndrome because a Dutch founder mutation is presumed to be the origin of TPS cases in the Southeast US, including Kentucky. To date only a single mutation, p.R674Q, in MYH8 has been reported to cause TPS. We report on two brothers with TPS syndrome who were found to have the same MYH8 mutation pR674Q previously described in other families . Patients description and Methods:These patients were two brothers of unrelated healthy parents. Physical examination at age 29 and 19 respectively revealed: a limited mouth opening reduced flexion of the trunk and hips, pseudocaptodactily and feet deformities. Genomic DNA was extracted, using standard protocols, from peripheral lymphocytes.The entire coding region of MYH8 was PCR amplified using previously reported primers [Veugelers et al., 2004] and HotstarTaq DNA polymerase (Qiagen, Inc., Valencia, CA) following the manufacturers recommendations. PCR products were purified by PCR purification Kit I (Eppendorf) Purified PCR products were sequenced using the ABI BigDye Terminator v.1.1 chemistry (Applied Biosystems, Inc., Foster City, CA) and an ABI 310 automated sequencer (Applied Biosystems, Inc.). Results:The presence of the p.R674Q (c.2021G>A) mutation was confirmed in both patients by restriction digestion with BsiW I (New England Biolabs,) Discussion: These results demonstrating that all cases of TPS studied to date are caused by an identical c.2021G>A mutation in MYH8 that causes the p.R674Q substitution, support the use of MYH8 testing in patient with a clinical diagnosis of TPS syndrome coming from different geographical areas.

Large family with an unusual SNRPN microdeletion : Interest of prenatal diagnosis. *J.-F. Vanbellinghen, L. Mutesa, A.-C. Hellin, S. Bertoli, G. Pierquin, V. Bours* Department of Human Genetics, University Hospital Liège, Belgium.

The Prader-Willi syndrome (PWS) is a complex genetic disorder due to the loss of function of genes in 15q11-q13 which are subject to genomic imprinting and expressed from the paternal allele only. The PWS is characterized by diminished fetal activity during pregnancy, severe hypotonia and feeding difficulties in early infancy, hypogonadism, small hands and feet, craniofacial dysmorphism, and hyperphagia leading to profound obesity in later enfancy or early childhood. Whereas the majority of PW patients have a de novo large deletion commonly encompassing the imprinting centre (IC), the SNRPN and UBE3 genes or due to maternal uniparental disomy (mUPD), the microdeletion of SNRPN are rare and appear to have a high recurrence risk. We report a large Caucasian family with a partial SNRPN gene microdeletion. To identify this deletion, we used methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) kit (MRC-Holland, Netherland) consisting of 43 probes detecting the copy number variation inside the Prader-Willi-Angelman critical region (15q11-q13). This deletion was identified in an affected child and in a fetus during a prenatal testing. The extensive familial study showed that several individuals were carriers of the microdeletion with high potential risk of PWS in inheritance. This report points out the interest of MS-MLPA technique versus Southern Blotting and importance of genetics counselling in family with SNRPN microdeletion.

Fasting status and dietary intake influence genetic associations for the APOA1/C3/A4/A5 gene cluster and triglyceride levels. *K. Brown[1], J. D. Smith[2], C. Shephard[2], M. Wong[2], M. J. Rieder[2], D. A. Nickerson[2], D. C. Crawford[1]* 1) Vanderbilt University, Nashville, TN; 2) University of Washington, Seattle, WA.

Increased triglyceride (TG) levels are associated with cardiovascular disease and are influenced by genes and environment. APOA1/C3/A4/A5 gene cluster variation has been associated with TG levels, and recent evidence suggests these variations interact with dietary intake of fatty acids. To expand these findings, we tested for an association the APOA1/C3/A4/A5 gene cluster with TG levels in 7157 participants in the population-based Third National Health and Nutrition Examination Survey (NHANES III). Seven tagSNPs were genotyped in 1236/1056 European-Americans (EA), 947/744 African-Americans (AA), and 1001/713 Mexican-Americans (MA) >17 years of age with fasting/non-fasting TG levels. All analyses were adjusted for total cholesterol, HDL, age, body mass index, and gender and stratified by race/ethnicity and fasting status. Among fasting participants, rs675 (p=0.04) and rs5104 (p=0.04) were associated with decreased and increased TG levels, respectively, in MAs while rs4520 (p=0.001) was associated with increased TG levels in both MAs and EAs (p=0.04). Among non-fasting participants, rs651821 (p=0.005), rs5104 (p=0.0002), rs5100 (p=0.02) and rs5092 (p=0.004) were associated with increased TG levels in MAs, rs5091 (p=0.010) was associated with decreased levels in MAs, and rs651821 (p=0.005) was associated with increased TG levels in AAs. Interaction terms for each SNP and dietary intake of fatty acids, protein, carbohydrates, and alcohol, expressed as the percentage of total energy intake, were tested for an association with ln(TG) levels using linear regression adjusting for covariates and marginal terms. Among fasting participants, 26 interaction terms were significant at $p<0.05$ and one term was significant at $p<0.0001$. Among non-fasting participants, 37 interaction terms were significant at $p<0.05$ and 8 were significant at $p<0.0001$. Both associated SNPs and interaction terms explained 2-12% of the variability in ln(TG) levels in our models. These results suggest fasting status and dietary intake influence the genetic associations we observed for TG levels in NHANES III.

Molecular diagnosis of Menkes disease: Genotype-phenotype correlation. *L. B. Møller, M. Mogensen, N. Horn*
Kennedy Center, Gl.Landevej 7, 2600 Glostrup, Denmark.

Menkes disease (MD) is an X-linked, multisystemic lethal disorder of impaired copper metabolism caused by mutations in the gene, ATP7A. ATP7A contains 23 exons and encodes a P-type ATPase of 1,500 aa. The ATP7A protein is a member of the CPx-type transmembrane ATPase family that performs ATP-driven translocation of metal cations across cellular membranes. The ATP7A protein is localised to the transGolgi membrane but translocated to the plasma membrane in response to increased copper concentration. In addition to the severe classic form of MD leading to death in early childhood, milder forms are observed in 5-10% of the patients. Occipital horn syndrome is the mildest allelic form of MD. To date more than 200 different mutations affecting ATP7A has been identified. These mutations include missense mutations, splice site mutations, small deletions/insertions and larger deletions/duplications of one or several exons. In order to correlate the genetic defect with the clinical expression we have investigated the effect of selected mutations on the resulting ATP7A transcript and on the resulting protein product. We will discuss the results of these investigations, and how these observations contribute to our knowledge about the function of the copper-transporting ATPase, ATP7A.

Stage-specific upregulation of epigenetic genes and IC-*SNRPN* transcripts during human spermatogenesis. *B. Horsthemke[1], M. Wawrzik[1], A. N. Spiess[2], K. Buiting[1]* 1) Institut für Humangenetik, Universitätsklinikum Essen, Germany; 2) Abteilung für Andrologie, Universitätskrankenhaus Hamburg-Eppendorf, Germany.

The imprinted domain in human 15q11-q13 is controlled by an imprinting center (IC), which overlaps the 5' part of the paternally expressed *SNRPN* gene. The gene has at least four alternative start sites, including *PWRN1*, which was previously reported to be a separate gene. The region also contains *C15orf2*, which is predominantly expressed in testis, but also in brain, where expression is imprinted, as well as the testis-specific gene *PWRN2*, which is transcribed from the opposite strand. It is unclear, when the IC-*SNRPN* locus is activated during spermatogenesis and which epigenetic factors are involved in this process. We have addressed this question by mining microarray-based gene expression data of testicular biopsies from men with different types of spermatogenic failure (complete tubular atrophy, Sertoli-cell only syndrome, arrest before meiosis, arrest at meiosis, arrest at round spermatides, and uniform hypospermatogenesis; n=27) and with normal spermatogenesis (n=8). The expression patterns of several spermatogenesis genes (*SYCP3*, *TNP1*, *PRM1* and *PRM2*) revealed that the data can be used to determine the stage-specific up- and downregulation of genes. The following epigenetic genes are upregulated before or at early meiosis: *CTCFL*, *SUV39H2*, *EZH2*, *BRDT*, *DNMT1* and a truncated form of *MBD2*. *MBD3L1* is upregulated at the round spermatid stage. Little or no germ cell-specific upregulation was seen for *MBD1*, the long form of *MBD2*, *MBD3*, *MBD3L2*, *MBD4*, *MBD5*, *MBD6*, *MECP2*, *DNMT2*, *DNMT3A*, *DNMT3B*, *DNMT3L*, *SUV39H1*, *HDACs*, and *CTCF*. This analysis shows that several epigenetic factors play an important role at specific stages of spermatogenesis. A complex pattern was seen for the IC-*SNRPN* locus: a hitherto unreported transcript was upregulated before or at early meiosis, whereas the *PWRN1-SNRPN* and *PWRN2* transcripts were upregulated at the round spermatid stage and expressed from the haploid genome. The *SNRPN* upstream transcripts may play a role in activating the paternal copy of 15q11-q13.

Identification of Notch1 as a potential biomarker in multiple myeloma patients by DNA Microarray. *S. Gupta, A. Dwivedi, S. Adhvaryu* Dept Pathology, Univ Texas Hlth SCSA, San Antonio, TX.

Multiple Myeloma (MM) is an incurable B-cell malignancy characterized by monotypic plasma cells in the bone marrow. MM is known to develop resistance to conventional therapies with average survival of 3-5 years. Understanding the molecular pathogenesis of MM will potentially improve the scope for designing newer strategies to treat MM. Currently, the underlying genetic changes contributing to initiation and progression of MM are not well known. Identifying these key molecular anomalies would help in its diagnosis and treatment. Our study attempted to identify biomarkers associated with development of MM. DNA was extracted from the cell pellets from the bone marrow specimens from MM patients received for cytogenetic analysis and an unpaired copy number variation (CNV) analysis was performed by using the Affymetrix SNP 6.0 chips. Several unique biomarkers were identified as amplified or deleted, including Notch1, which was found to be amplified in more than 75% of the MM samples analyzed. Notch signaling plays a key role in the development and differentiation of various hematopoietic lineages. Notch signaling receptors are expressed in hematopoietic stem cells and its ligands are found to be expressed in bone marrow stroma. This provides a microenvironment for maintenance of hematopoietic stem cells and their development/ differentiation into other blood cell lineages. Role of Notch signaling in the pathogenesis of T-cell leukemia is well established. However, role of Notch signaling in development of B-cell neoplasm such as MM is not clear. Notch1 signaling, via its ligand Jagged1, has been shown to promote tumor cell growth and survival. Recent publications emphasized direct role of Notch signaling in the pathogenesis of MM. Other findings showing inhibition of Notch signaling induces apoptosis of MM cells and enhanced sensitivity to chemotherapy further substantiates our hypothesis. Our findings clearly underscore the role of Notch signaling pathway in MM. Further investigation in this area could help in establishing Notch1 as an important biomarker of MM and in designing specific treatment of MM.

Identification of a case of Limb-Girdle Muscular Dystrophy Caused by Uniparental Disomy of Chromosome 4. *R. E. Pyatt, M. Hart-Kothari, J. Frick, D. Lamb Thrush, M. Pastore, C. Shilling, X. Q. Rosales, J. Mendell, J. M. Gastier-Foster* Nationwide Childrens Hospital, Columbus, OH.

Limb-Girdle Muscular Dystrophy (LGMD) is a group of disorders which present with weakness and wasting of the voluntary muscles mainly around the pelvic and shoulder regions. LGMDs are caused by defects in multiple genes and can be autosomal dominant (type 1) or autosomal recessive (type 2). Further subdivisions are based on presentation, onset of weakness, and the involvement of specific muscle groups. LGMD2E is characterized by autosomal recessive inheritance, mutations in the beta-sarcoglycan gene (SGCB), and severe, childhood-onset of muscle weakness. The proband was first evaluated at age 9 with hip stiffness, difficulty climbing stairs, abnormal gait when running, and enlarged calf muscles. During routine mutation analysis of the SGCB gene on chromosome 4, the proband presented as apparently homozygous for a single base substitution in exon 4 (452C>G [T151R]) with no other alterations in the coding regions or exon-intron boundaries observed. Subsequent analysis of the SGCB gene in the probands parents revealed the mother to be a heterozygous carrier of the same mutation while no pathologic alterations were found in the father's gene. These results suggested the presence of a large deletion including exon 4 on the paternal allele, a de novo mutation, uniparental disomy, or nonpaternity. Uniparental disomy is the inheritance of both copies of a chromosome pair from a single parent. Microsatellite analysis was conducted using the PowerPlex 16 identity panel to exclude nonpaternity. To test for uniparental disomy of chromosome 4, the alleles for 10 additional microsatellite markers were compared between the proband and parents. Loci on chromosome 4 demonstrated exclusive inheritance of maternal alleles with no paternal contribution. These results represent the first reported case of LGMD caused by UPD.

Evaluation of genes responsible for homocysteine metabolism in a pseudoexfoliation glaucoma case control sample. *J. L. Wiggs[1], B. Fan[1], T. Li[1], C. Grosskreutz[1], L. Pasquale[1], T. Chen[1], D. Rhee[1], E. DelBono[1], J. L. Haines[2]* 1) Dept Ophthalmology, Harvard Medical Sch, MEEI, Boston, MA; 2) Center for Human Genetics Research, Vanderbilt School of Medicine, Nashville, TN.

Glaucoma is a genetically and phenotypically heterogeneous disorder that causes irreversible degeneration of the optic nerve and is a leading cause of blindness worldwide. Pseudoexfoliation glaucoma (PXF) and primary open-angle glaucoma (POAG) are the two most common forms of the disease. Recently, SNPs in LOXL1, coding for a protein that participates in elastogenesis, were significantly associated with PXF, however the risk haplotype is also prevalent in control samples, indicating that additional genetic factors and/or environmental exposures could contribute to this complex disease. Previous studies have indicated that plasma levels of homocysteine are elevated in patients with PXF suggesting that the genes determining homocysteine levels may be good candidates for secondary genetic factors. In the present study, we evaluated five genes that participate in homocysteine metabolism (CBS, MTHFD1, MTHFR, MTR, MTRR) in 215 PXF patients and 181 controls. Genotypes for 9 SNPs (including nonsynonomous SNPs with possible biologic effect) were obtained using either the TaqMan assay or direct genomic sequencing. Single-SNP association analysis was performed using SAS statistical software and pairwise-SNP interaction analysis was performed using PLINK. None of these SNPs were independently associated with PXF and only a weak interactive effect was found for one SNP in MTRR (rs161870, L206L) with LOXL1 ($p = 0.074$). These results suggest that this group of genes responsible for homocysteine metabolism does not contribute significantly to PXF. Future studies include investigation of additional SNPs and associated haplotypes in this group of genes as well as SNPs and haplotypes associated with genes coding for proteins that contribute to the maintenance of elastic fibers and composition of the extracellular matrix that are also excellent candidates for additional genetic factors that could contribute to this common blinding disease.

FOLLOW UP OF FIVE LINKAGE PEAKS FOR AUTISM IN EXTENDED UTAH KINDREDS. *K. Allen-Brady[1], N. Matsunami[2], J. Stevens[2], L. Baird[2], R. Robison[3], D. Cannon[3], J. Miller[3], T. Leppert[2], C. Pingree[3], M. F. Leppert[2], W. McMahon[3], H. Coon[3]* 1) Gen Epidemiology, Univ Utah, Salt Lake City, UT; 2) Human Genetics, Univ Utah, Salt Lake City, UT; 3) Psychiatry, Univ Utah, Salt Lake City, UT.

Autism is a complex, early-onset behavioral disorder characterized by impairments in social interactions and communication, and by repetitive and stereotyped behaviors and interests. Recent studies have revealed a highly complex genetic landscape for autism spectrum disorders, with many potential genes of varying and as yet unknown importance. Extended pedigree studies may play an important role in clarifying genetic findings. Our previous genome scan in a single six-generation extended Utah pedigree identified three linkage regions meeting criteria for genome-wide significance at 3q13.2-q13.31, 3q26.31-q27.3, and 20q11.21-q13.12. Two additional regions (7p14.1-p11.22 and 9p24.3) met criteria for suggestive significance. We have followed up these regions in our sample of 33 families with 125 affected subjects (total n=386). Evidence for linkage appears to be supported in this expanded set of families for the regions on chromosomes 20q11.2-q13.1 (maximum non-parametric score = 3.51, p=0.00023 at 64.8 cM) and 3q26-q27 (maximum non-parametric score = 3.77, p=0.000084, at 184.6 cM). Other regions showed less support in other kindreds, and may indicate susceptibility genes unique to the original pedigree.

A genome-wide quantitative association analysis of plasma homocysteine levels in schizophrenic patients. *S. de Jong[1], JW. Muntjewerff[2], M. Hoogendoorn[3], R. S. Kahn[3], R. A. Ophoff[1,4]* 1) Complex Genetics Section, DBG-Department of Medical Genetics, University Medical Centre, Utrecht, The Netherlands; 2) GGz Nijmegen, Mental Health Institute, Nijmegen, The Netherlands; 3) Department of Psychiatry, Rudolf Magnus Institute of Neuroscience, University Medical Centre Utrecht, Utrecht, The Netherlands; 4) Rudolf Magnus Institute of Neuroscience, University Medical Centre Utrecht, The Netherlands.

In addition to being an independent risk marker for cardiovascular disease, increased plasma total homocysteine increased levels have also been related to Alzheimers disease and schizophrenia. It has been discovered that homocysteine elicits a DNA damage response in neurons. This suggests a mechanism by which homocysteine may contribute to the pathogenesis of neurodegenerative disorders. Many environmental factors such as low folate or cobalamine intake and genetic factors influence the homocysteine concentration in plasma. A common variant in the MTHFR gene has been clearly associated with elevated homocysteine levels and increased schizophrenia risk. Other genetic variants that modulate homocysteine levels are genes of interest to study in relation to the risk of schizophrenia. For this study, plasma homocysteine levels were determined in 70 schizophrenic patients. meeting DSM-IV criteria for schizophrenia. All subjects were unrelated and of Dutch Caucasian descent. Sample collection was carefully done in uniform conditions between subjects. Genome-wide SNP data was available for these subjects. A genome wide quantitative association analysis was performed using PLINK including haplotype analysis. Age and gender were taken as covariates and significance of results was determined using permutations and false discovery rate. Results reveal no significant genome wide association. However, considering a priori knowledge, the common variant in the MTHFR gene was also found to be significantly associated with homocysteine levels. Lager sample size and inclusion of a control group would increase statistical power to detect associated SNPs. Results of this study can help in unraveling the genetic regulation of homocysteine and the relationship with schizophrenia.

AML with multiple copies of unrearranged MLL gene: a distinct cytogenetic entity. *G. Sun[1], L. Montella[1], C. Barnabe[1], W. Henderson[1], J. Tripodi[1], H. Tamim[2], B. Saidman[3], R. Moser[4]* 1) Genzyme Genetics, New York, NY; 2) Atlanta Cancer Care, Decatur, GA; 3) Wyoming Valley Health Care Systems, Wilkes Barre, PA; 4) St. Francis Medical Center, Trenton, NJ.

The MLL gene at 11q23 is often involved in acute leukemias, mostly present as reciprocal translocations, as partial tandem duplication (PTD) in trisomy 11 or a normal karyotype, and also as double minutes (dmin) or homogenously staining region (hsr). There have been occasional case reports on unrearranged MLL gene in AML. We recently studied four such cases. We believe that this category of gene copy number alteration is not uncommon in AML, particularly in treatment-related AML and that it should bring our attention when analyzing the karyotypes that show normal chromosomes 11 and derivative chromosomes with unknown material attached. FISH is essential for confirmation. As MLL gene copy alteration plays a pivotal role in leukemogenesis and portends a poor clinical outcome, it is important to recognize this distinct cytogenetic entity in AML. Patient JR is a 63 year old female with ovarian cancer post chemotherapy before the diagnosis of AML M4. Cytogenetics showed der(15)t(11;15),der(22)t(11;22),der(13)t(11;13),der(22)t(11;22). Patient GJ is a 68 year old female diagnosed as AML M7 arising from an MPD/MDS, showing 2 unrelated clones: one with del(5)(q15q31)and another complex with der(14)t(11;14)x2,der(21)t(11;21). Patient JB is an 86 year old male diagnosed as AML M5b with a history of thrombocytopenia and suspected T-cell proliferative disorder. Cytogenetics revealed 2 unrelated abnormal clones: one with der(7)t(7;11)(q32;q21)x2 and another +8. Patient EC is a 97 year old female with a diagnosis of AML on peripheral blood (subtype unknown). Cytogenetics showed der(13)t(11;13). All MLL genes were intact without translocation or amplification using a break-apart probe (Vysis). Interestingly, three of the four patients showed segmental translocations to each of the acrocentric chromosomes with the same breakpoints q21 on chromosomes 11 and p13 on acrocentric chromosomes. The homologous DNA sequences in the breakpoints may play a role in mediating the translocations.

Genealogical relationships and genetic distance between two tone language-speaking native communities in the Mexican states of Oaxaca & Hidalgo. *A. Sanchez-Boiso[1], R. Peñaloza-Espinosa[2], R. Sánchez-Urbina[1], E. Castro-Sierra[3], RI. Ortiz-de Luna[1], L. Buentello-Malo[4], F. Salamanca-Gómez[2], R. Cerda-Flores[5], V. Morán-Barroso[1]* 1) Dept Genetics, Hosp Infantil Mexico Fed Gomez, Mexico City, Mexico; 2) UIMGH, Hospital de Pediatría CMNSXXI; 3) LPAFA, HIMFG; 4) IIA, UNAM; 5) Genética de Poblaciones y Bioinformática, CIBIN-IMSS.

Mexico has a diversity of native groups. There are relatively few mtDNA studies of native Mexican populations in regard to their mitochondrial haplogroup profiles associated to their linguistic characteristics. Relating to populations in the American continent, haplogroups A, B, C & D are the most frequently reported. In the present study, genetic distance between two tone language-speaking communities, Zapotec-speaking, Juchitán, Oaxaca, and Otomi-speaking, San Antonio el Grande, Hidalgo, is analyzed based on their genealogical relationship. Thirty-eight samples were studied in San Antonio el Grande, and 38 in Juchitán. DNA was isolated and PCR of Amerindian haplogroups carried out using either enzymatic restriction (haplogroups A, C & D) or polyacrylamide gel (haplogroup B) techniques. Finally, a main component statistical analysis was undertaken to determine similarities or differences between populations based on haplogroup frequency. Analysis of frequencies of the 4 haplogroups provided evidence of both San Antonio & Juchitán showing a greater frequency of haplogroups A and B and a lesser one of haplogroups C and D. In each population, there was a minority of individuals who did not possess the 4 Amerindian haplogroups studied. These frequencies were analyzed using Miller RxC method, employing 50,000 tests and confirming results with main component analysis (SPSS v15.0). Results obtained indicate that the population from San Antonio and the population from Juchitán apparently belonging to the same linguistic family, and notwithstanding the considerable geographic distance (ca. 500 mi.) separating either, with non-tone language-speaking groups inserted between them, share similar frequencies of the 4 haplogroups investigated.

Association Studies of 22 Candidate SNPs with Late-Onset Alzheimers Disease. *J. A. Figgins[1], R. L. Minster[1], F. Y. Demirci[1], S. T. DeKosky[2], M. I. Kamboh[1]* 1) Dept Human Genetics, Univ Pittsburgh, Pittsburgh, PA; 2) Dept Neurology, Univ Pittsburgh, Pittsburgh, PA.

Alzheimers disease (AD) is a complex and multifactorial disease with the possible involvement of several genes. With the exception of the APOE gene as a susceptibility marker, no other genes have been shown consistently to be associated with late-onset AD (LOAD). A recent genome-wide association study of 17,343 gene-based putative functional single-nucleotide polymorphisms (SNPs) found 19 significant variants, including 3 linked to APOE, showing association with LOAD (Hum. Mol. Genet. 2007; 16:865-73). We set out to replicate the 16 new significant associations in a large case-control cohort of American Caucasians. Additionally, we examined six variants present in positional and/or biological candidate genes for AD. We genotyped the 22 SNPs in up to 1,009 Caucasian Americans with LOAD and 1,010 age-matched healthy Caucasian Americans, using 5' nuclease assays. We did not observe a statistically significant association between the SNPs and the risk of AD, either individually or stratified by APOE. However, one SNP on chromosome 17q13 revealed significant association with age-at-onset of AD ($p = 0.00196$) and two linked SNPs on chromosome 1q21 were associated with disease duration ($p = 0.006$; $p = 0.0014$). The association of studied variants with LOAD risk was not statistically significant in our sample, but may be relevant to some quantitative traits related to AD.

Gene expression response to hydrogen peroxide identifies possible new components of the response to reactive oxygen species. *K. A. Chapman[1], K. G. Ewens[2], R. S. Spielman[2]* 1) Division of Genetics, The Children's Hospital of Philadelphia, Philadelphia, PA; 2) Department of Genetics, University of Pennsylvania, Philadelphia, PA.

Reactive oxygen species (ROS) are known to influence key cellular processes including cellular stress, apoptosis, and immunologic response, and are implicated in human diseases including Alzheimers disease, ischemia-reperfusion injury, and atherosclerosis. Our goal was to survey expressed genes for transcriptional response to ROS produced by hydrogen peroxide (H_2O_2). Lymphoblastoid cell lines (LCLs) from four HapMap individuals were treated with 9, 90, or 900 M H_2O_2, for 4, 12, 24, or 48 h. Controls were grown for the same periods without exposure to H_2O_2. The mRNA from the cells was pooled within each of the 4 doses x 4 durations, and expression profiles were obtained with the Affymetrix Human Gene 1.0 ST array. The gene expression fold change was then calculated for each treatment. The largest number of transcripts (88) with expression responses greater than 3-fold was found in the LCLs treated with 900 M for 4 hours, so we restricted further analysis to this group.

We found strong expression changes in several genes previously known to respond to ROS. For example, three genes responsive to p53 were activated: GDF15 (fold change compared to untreated: 4.7), CDKN1A (3.1), and PPM1D (3.5). Other examples are the ROS-responsive gene, HMOX1 (2.9) and apoptosis related G-protein coupled receptor GPR109B (PUMA-G, 6.0). In addition, we identified several gene families that have not been previously characterized as components of the ROS response. These include the small RNAs SCARNA9 (6.3 fold change) and SNORD 116-6 (5.02), the miRNA regulator DND1 (4.34), and the zinc finger binding protein ZNRD1 (4.10). Thus our approach allows further studies on previously known genes, and also identifies new ROS-responsive genes, which will expand the understanding of the biological effects of ROS exposure.

Array comparative genomic hybridization detects genomic imbalances in samples from stillborn infants. *G. Raca[1,2], A. Artzer[4], L. Thorson[4], J. Shu[1], S. Huber[1], J. S. Laffin[1], P. Modaff[3], R. M. Pauli[3]* 1) UW Cytogenetic Services, State Laboratory of Hygiene, Madison, WI; 2) Department of Pathology, UW-Madison; 3) Wisconsin Stillbirth Service Program (WiSSP), Clinical Genetics Center, UW-Madison; 4) Department of Medical Genetics, UW-Madison.

Current testing methodologies fail to explain late pregnancy loss (stillbirth) in up to 60% of cases, resulting in inaccurate recurrence risk assessment and limited clinical management in subsequent pregnancies. Array comparative genomic hybridization (aCGH) has been successfully applied to samples from early pregnancy loss, but has rarely been used in evaluation of stillbirth. We hypothesized that aCGH could establish etiologic diagnosis in a much higher proportion of stillborn infants than could classic cytogenetics, by detecting both gross and submicroscopic deletions and duplications, and by not requiring viable tissue for growth in vitro. We analyzed 15 frozen tissue samples from stillborns with multiple congenital anomalies, obtained through the Wisconsin Stillbirth Service Program (WiSSP). For all 15 samples results of classic cytogenetic analysis were either normal or unobtainable. Samples were tested in a blinded fashion using commercially available 1Mb BAC Arrays (PerkinElmer). aCGH detected two abnormalities, trisomy 21 and an unbalanced translocation between chromosomes 3 and 10. With the detection rate of more than 13%, our preliminary results support the clinical value of aCGH testing in stillbirth. The information about each of the two detected abnormalities would have been helpful in counseling of the parents, had it been available at the time of pregnancy loss. aCGH analysis should be considered instead of or in addition to karyotyping for routine diagnostic evaluation of late intrauterine death. aCGH could also be a valuable research tool to 1) examine the etiologic role of submicroscopic deletions and duplications in intrauterine death, 2) identify critical regions and candidate genes for specific developmental anomalies present in tested stillborns, and 3) identify candidate chromosomal regions that are critically involved in survival through late gestation.

Expectations for Research Result Disclosure: A Qualitative Analysis of Public Opinion Regarding a Proposed National Biobank. *J. Murphy[1], J. Scott[1], D. Kaufman[1], G. Geller[2], L. LeRoy[3], K. Hudson[1]* 1) Genetics & Public Policy Ctr, Johns Hospkins Univ, Washington, DC; 2) Johns Hopkins University, Baltimore, MD; 3) Abt Associates, Cambridge, MA.

Introduction: The National Institutes of Health and other federal health agencies are considering establishing a national biobank to study of the roles of genes and environment in human health. A pilot public engagement study was conducted to assess public attitudes and concerns about the proposed biobank, including the expectations for return of individual research results. Methods: A total of 141 adults of different ages, incomes, genders, ethnicities, and races participated in 16 focus groups in six locations across the country. Focus group members were queried about their views and preferences relating to return of five different types of research results: (1) identification of a gene variant that increases the risk for asthma (treatable condition), (2) identification of an environmental factor that increases risk for asthma (environmental factor), (3) identification of a gene variant that increases risk for Alzheimer disease (untreatable condition), (4) identification of a gene variant with a higher prevalence in a racial or ethnic group, and (5) identification of a gene variant of unknown significance. Results: Focus groups participants voiced a strong desire to be able to access individual research results, regardless whether or not an intervention was available. Recognizing the wide range of possible research results from a large cohort study, they repeatedly and spontaneously suggested that cohort study participants be given on-going choices, often using Internet analogies such as setting preferences or filters. Conclusion: Our focus group analysis indicates that access to individual research results was viewed as a valuable incentive for participating in the proposed biobank. The project described was supported by Grant Number U01HG004206 from the National Human Genome Research Institute.

Prenatal diagnosis of anophthalmia/microphthalmia by ultrasound: Recommendations for further testing and counseling. *A. Schneider, T. Bardakjian* Dept Genetics, Albert Einstein Medical Ctr, Philadelphia, PA.

Anophthalmia and Microphthalmia (referred to as A/M) may involve one or both eyes, and may occur as an isolated birth defect or with other anomalies (part of a syndrome). A/M is heterogeneous and rare with an incidence of about 1 in 10,000. Ultrasound diagnosis of anophthalmia/microphthalmia is possible in the second trimester, usually 20 weeks gestation or after. Transvaginal ultrasound can detect A/M as early as 14 weeks. There is a paucity of information in the medical literature regarding further work-up and management of a fetus diagnosed with A/M. The A/M registry at Albert Einstein Medical Center has been collecting clinical data in a registry for the past 13 years and facilitates eye development gene research for those affected with A/M. Based on the experience and data analysis of over 300 cases of A/M, recommendations for the work-up of prenatally diagnosed A/M will be discussed. Newer eye development genes like SOX2 are now known to be the etiology of A/M in 15-20% of cases of bilateral A/M and a smaller percentage of unilateral A/M. From the registry data, about 11% of cases of A/M are due to chromosome abnormalities. A systematic review of tiers of testing including cytogenetic, molecular and additional ultrasound examinations will be discussed. Outcomes based on registry data can be used as an additional tool to help counsel families about this rare disorder.

***Creld1* mutations increase susceptibility to congenital heart defects in Down syndrome.** *S. M. Cherry[1], G. T. Fouad[2], C. L. Maslen[2], R. H. Reeves[1]* 1) Physiology, Johns Hopkins University, Baltimore, MD; 2) Molecular and Medical Genetics, Oregon Health & Science University, Portland, OR.

Down syndrome (DS) affects approximately 1 in 700 newborns and comprises a broad range of phenotypes, including congenital heart defects (CHD). Approximately 20% of DS individuals have an atrioventricular septal defect (AVSD), an incidence that is 2000-fold higher than in the general population. Dosage imbalance for chromosome 21 clearly contributes to heart defects in DS, but other genetic or environmental factors must contribute as well.

A recent study identified polymorphisms in the *CRELD1* locus that are associated with AVSD in individuals with DS (Maslen *et al*., 2006). To establish a biological basis for this interaction, we have initiated studies of CHD in animal models of Down syndrome. First, mouse gene-targeting was used to create a null allele of *Creld1* (*Creld1*$^{-}$); homozygous *Creld1*$^{-/-}$ die by embryonic day 12.5 and exhibit abnormal heart development. *Creld1*$^{+/-}$ mice, however, appear phenotypically normal, indicating that loss of a single allele does not cause heart defects. We then introduced the *Creld1* null allele into Ts65Dn mice, which have segmental trisomy for chromosome 16, representing nearly half of the human chromosome 21 orthologs. About 15% of newborn Ts65Dn have defects of the outflow tract, with occasional errors in development of the secondary atrial septum; overall CHD is less severe than in humans. Initial data indicate that more than 50% of mice that are both trisomic and heterozygous for the *Creld1* mutation (Ts65Dn, *Creld1*$^{+/-}$) display septal defects at birth, including both ASD and VSD. These data support the hypothesis that *Creld1* acts as a modifier of CHD in individuals with DS. We are currently crossing this mutation into mice with smaller segmental trisomies to localize the orthologous region of chromosome 21 responsible for this interaction.

On the replication of genetic associations: Timing can be everything! *J. Lasky-Su[1], H. Lyons[2], V. Emilsson[3], I. Heid[4,8], C. Molony[3], B. Raby[1], E. Silverman[1], D. Levy[5], M. McQueen[6], N. Laird[7], C. Papoutsakis[11], G. Dedoussis[11], C. O'Donnell[5], H. Wichmann[4,8], J. Celedon[1], E. Schadt[3], J. Hirschhorn[2,9], S. Weiss[1], K. Stefansson[10], C. Lange[1,7]* 1) Channing Laboratories, Dept Med, Brigham & Womens Hosp, Boston, MA; 2) Divisions of Genetics and Endocrinology, Program in Genomics, Childrens Hospital, Boston, MA; 3) Rosetta Inpharmatics, LLC, a wholly owned subsidiary of Merck & Co., Inc., Seattle, WA; 4) GSF-National Research Centre for Environment and Health, Institute of Epidemiology, Neuherberg, Germany; 5) National Heart, Lung, and Blood Institute and its Framingham Heart Study, Framingham, MA; 6) University of Colorado at Boulder, Boulder, CO; 7) Harvard School of Public Health, Boston, MA; 8) Institute of Medical Informatics, Biometry, and Epidemiology, Ludwig-Maximilians-University, Munich, Germany; 9) Program in Medical and Population Genetics, Broad Institute of Harvard and MIT, Cambridge, MA; 10) deCode Genetics, Inc., IS-101 Reykjavik, Iceland; 11) Department of Nutrition and Dietetics, Harokopio University, Athens 17671, Greece.

The failure to replicate genetic association findings is most commonly believed to be attributable to insufficient statistical power, population stratification, heterogeneity or environmental influences. Another potential cause for non-replications is a genetic effect that varies by age. If not taken into account during the design and the analysis of the study, age-varying genetic associations can be the reason for non-replication. Using the 100K SNP scan of the Framingham Heart Study (FHS), we identify an age-varying association between a SNP in ROBO1 and obesity. Based on these data, we hypothesized that there is an age-gene interaction. This finding was followed-up in 8 independent samples comprised of 13,584 individuals. The association replicates in 5 of the 8 studies, showing an age-dependent relationship ($p = 3.92 \times 10^{-9}$). This study illustrates that if the specifics of age-varying genetic effects are not considered in the selection of both the follow-up samples and in the statistical analysis, important genetic associations may be missed.

Novel REEP1 isoforms and their impact on subcellular localization and genetic testing. *S. Zuchner[1], J. Huang[1], T. Deconinck[2,3], J. Price[1], M. Pericak-Vance[1], G. Wang[1], P. de Jonghe[2,3], G. Montenegro[1]* 1) Human Genomics, Miami Institute for Human Genomics, University of , Miami, FL; 2) Molecular Genetics Department, Flanders Interuniversity Institute of Biotechnology, University of Antwerp, Antwerp, Belgium; 3) Division of Neurology, University Hospital Antwerpen, Antwerpen, Belgium.

Background: Recently we have shown that mutations in REEP1 cause hereditary spastic paraplegia (HSP) type 31. REEP1 is now considered the third most common HSP gene and genetic testing is frequently carried out according to the reported gene annotation. REEP1 is a protein of unknown function, but we have shown in-vitro its mitochondrial localization. Methods: We performed extensive cDNA studies, including exon walking, RACE-PCR, and studying intragenic conserved non-coding elements. Sequencing analysis was carried-out at a large sample of HSP index patients. In-vitro experiments were performed at transiently transfected cell lines. Results: We further studied the genetic structure of REEP1 and identified additional exons and alternative isoforms. In screening a large collection of HSP patients we identified a novel mutation in one of the new REEP1 exons. Interestingly, at least one new isoform is missing the mitochondrial targeting signal at the N-terminal end of REEP1. In cell culture experiment we are evaluating the subcellular localization of the main REEP1 isoforms. Discussion: The identification of additional coding exons inREEP1 has important consequences for genetic testing in SPG31. Future genetic tests should include these novel coding exons. The subcellular localization of REEP1 could be significantly different in the expressed isoforms. How this relates to the pathology of SPG31 will be an important part of future functional studies.

Nuclear APC in DNA repair. *M. Zeineldin, J. Cunningham, KL. Neufeld* Molec Bioscience, Univ Kansas, Lawrence, KS.

Mutation of the Adenomatous polyposis coli gene (Apc) is an early step in progression of about 80% of colorectal cancers. The product of the Apc gene, APC, is a large protein implicated in many cellular functions including cellular proliferation, migration, adhesion, and chromosome segregation. APC shuttles between the cytoplasm and nucleus using several nuclear localization signals (NLS) and nuclear export sequences (NES). Our lab has a long standing interest in nuclear functions of APC. To facilitate our studies, we have developed mice that are defective in nuclear import of APC because of mutations in two nuclear localization signals in APC [APC(mNLS)]. Others have suggested that APC participates in long-patch base excision repair (LP-BER). In the nucleus, oxidation, reduction or alkylation of DNA leads to modified bases which are repaired using BER. Using our mouse model as well as cultured colon cell lines, we have further explored the potential role of nuclear APC in DNA repair and examined the regulatory mechanisms that control APC stability. We will provide evidence that APC(mNLS) mouse embryonic fibroblasts (MEFs) have a reduced ability to repair DNA damaged by alkylating agents compared to MEFs with wild-type APC. In addition, we have evidence to suggest that hsp70 participates in stabilization of APC by preventing it from being degraded through the ubiquitin-proteasome pathway.

Genetic ancestry and risk of breast cancer among US Latinas. *L. Fejerman[1], E. M. John[2], S. Huntsman[1], K. Beckman[3], S. Choudhry[1], E. Perez-Stable[1], E. Gonzalez Burchard[1], E. Ziv[1]* 1) Dept Med, Univ California, San Francisco, CA; 2) Northern California Cancer Center, Fremont, CA; 3) Children's Hospital Oakland Research Institute, Oakland, CA.

Background: US Latinas have a lower incidence of breast cancer compared to non-Latina White women. This difference is partially explained by differences in the prevalence of known risk factors. Genetic factors may also contribute to this difference in incidence. Latinas are an admixed population with most of their genetic ancestry from Europeans and Indigenous Americans. We used genetic markers to estimate the ancestry of Latina breast cancer cases and controls and assessed the association with genetic ancestry, adjusting for reproductive and other risk factors. Methods: We typed a set of 106 ancestry informative markers (AIMs) in 440 Latina women with breast cancer and 597 Latina controls from the San Francisco (SF) Bay area and estimated genetic ancestry using a maximum likelihood method. Odds ratios (OR) and 95% confidence intervals (CI) were estimated using logistic regression with known risk factors included as covariates. Results: Higher European ancestry was associated with increased breast cancer risk (OR=4.17; 95% CI: 2.13-8.17, p0.001). When known risk factors and place of birth were adjusted for, the association with European ancestry was attenuated but remained statistically significant (OR=2.58; 95% CI: 1.22-5.45, p=0.013). Conclusions: Among Latinas in the SF Bay Area, women with higher European ancestry are at higher risk for breast cancer, even after adjustment for known risk factors. Further work is needed to determine if the association is due to genetic differences between populations or possibly due to environmental factors not measured.

Effect of an educational consent video on family study research participants' preferences for future use of DNA.

S. Lewis[1], K. Spates[1], G. D. Hughley[2], C. Burant[3], P. M. Marshall[2], G. L. Wiesner[1,4] 1) Dept Genetics, Case Western Reserve University, Cleveland, OH; 2) Dept Bioethics, Case Western Reserve University, Cleveland, OH; 3) Frances Payne Bolton School of Nursing, Case Western Reserve University, Cleveland, OH; 4) Center for Human Genetics, University Hospitals Case Medical Center, Cleveland, OH.

Genetic researchers and public health officials working to establish large human DNA repositories for long-range health research struggle with privacy issues related to sharing of DNA when possible future research directions are unknown. Access to DNA samples from repositories can improve genetic research efficiency by reducing the need for re-collection of samples. However, to protect autonomy and privacy of research participants, guidelines for informed consent may ask that participants indicate their preference for future use of DNA. We previously reported on future use preferences of 2226 genetic study participants in which 49% chose UNRESTRICTED future use if identifiers were removed; 46% chose RECONTACT before allowing use; and 4% chose NO FUTURE USE. To assess the effect of the consent process on choices we examined preferences among a sub-group of 240 African American participants recruited as part of a multi-center study of informed consent. Participants were randomized to view either a 10-minute "educational" or "placebo" video during the informed consent process. In the educational arm, 117 participants viewed a potential participant and recruiter engaged in an informed consent conversation. In the placebo arm, 123 participants viewed nature footage. Surprisingly, significant differences in preferences for future use between the two groups were found (p=.007), with those who viewed the educational video (17%) less likely to choose unrestricted future use than those who viewed the placebo video (35%). Differences persisted when controlling for demographic factors. Understanding the impact of viewing an educational video on preferences for future use of DNA may help researchers design tools to inform and educate participants about open ended uses of DNA collections and data sets in genetic studies.

PREIMPLANTATION HLA TYPING FOR STEM CELL THERAPY OF GENETIC DISORDERS. *A. Kuliev, S. Rechitsky, T. Sharapova, I. Barsky, O. Verlinsky, I. Tur-Kaspa, Y. Verlinsky* Reproductive Genetics Institute, Chicago, IL.

Preimplantation HLA typing is part of our preimplantation genetic diagnosis (PGD) program for single gene disorders, which has presently been performed for 178 different genetic conditions in 1462 cases, yielding the birth of 500 unaffected children. Preimplantation HLA typing allows the stem cell treatment of affected siblings for whom no other treatment is available. This was applied not only together with PGD, but also for sole purpose of the pre-selection of the HLA matched embryos, which become of special value with progress in stem cell transplantation treatment of an increasing number of severe congenital and acquired bone marrow disorders. Overall we performed preimplantation HLA typing in 242 cases, representing the worlds largest series, yielding birth of 37 healthy HLA matched babies. A total of 153 cases were performed in combination with PGD for different genetic disorders (thalassemia, sickle cell disease, Fanconi anemia (FA), Wiscott-Aldrich syndrome (WAS), X-linked adrenoleukodystrophy (X-ALD), X-linked hyper hyperimmunobulin M syndrome (HYGM), X-linked hypohidrotic ectodermal displasia with immune deficiency (HED-ID), Krabbe disease and inherited form of Diamond-Blackfan anemia (DBA), involving the pre-selection of unaffected children who were also HLA identical to the affected sibling. Successful stem cell transplantation treatment with the use of stem cells obtained from PGD children has been achieved in more than a dozen patients with genetic and acquired diseases, including thalassemia, FA, DBA, X-linked HYGM and HED-ID.

Cytogenetic studies of meiotic recombination in human females. *T. Hassold[1], T. Naluai-Cecchini[2], S. Cherry[1], T. Hansen[1], K. W. Broman[3], E. Cheng[2]* 1) Sch Molecular Biosci, Washington State Univ, Pullman, WA; 2) Dept Ob/Gyn, University of Washington, Seattle, WA; 3) Department of Biostatistics and Medical Informatics, University of Wisconsin-Madison, Madison, WI.

Until recently, almost all studies of meiotic recombination in humans have been based on genetic linkage analyses. However, the application of immunofluorescence methodology now allows us to directly visualize meiotic exchanges; i.e., by analyzing cross-over associated proteins (e.g., MLH1) in pachytene stage spermatocytes and oocytes, we can determine the number and distribution of exchanges in individual gametes. Utilizing this approach, several groups have now initiated studies of human female meiosis, but in most of these the focus has been on meiotic process, and not specifically on recombination. Thus, we recently initiated studies of a series of human fetal ovarian samples, aimed at evaluating the utility of MLH1 as a marker of crossing-over in females and asking whether chromosomes without cross-overs and sub-optimal cross-over patterns - situations thought to predispose to human nondisjunction -- are, indeed, a feature of human female meiosis. In this report we summarize results on an initial series of 411 prophase oocytes from 12 fetal ovarian samples. Our results indicate that MLH1 foci are a useful marker of cross-overs in females as well as males and that, as in males, there is significant among-individual variation in the levels of recombination. However, we also identified important temporal differences between human males and females in the way in which MLH1 loads onto the synaptonemal complex, demonstrating sex-specific variation in the control of the recombination pathway. In analyses of non-disjunction prone chromosomes, we identified a high proportion of exchangeless chromosomes 21 and 22, as well as an increased proportion of distal exchanges on chromosome 16. These observations are consistent with linkage analyses of human trisomies, confirming assumptions of the role of recombination in predisposing to human nondisjunction.

Gender differences in UGT2B17 expression and activity. *C. J. Gallagher, R. M. Balliet, J. E. Muscat, P. Lazarus*
Departments of Pharmacology and Public Health Sciences, Penn State College of Medicine, Hershey, PA.

UGT2B17 is a phase II metabolizing enzyme involved in the glucuronidation of androgens as well as exogenous compounds including the tobacco carcinogen 4 (methylnitrosamino) 1 (3 pyridyl) 1 butanol (NNAL) and cancer treatment drugs including suberoylanilide-hydroxamic-acid (SAHA). UGT2B17 is expressed in many tissues including liver, lung, and prostate and is down-regulated by androgens in prostate tissue. Due to the fact that this gene metabolizes and is regulated by androgens, we hypothesized that there may be gender differences in the expression and activity of this gene. We performed reverse-transcription PCR on 77 human liver RNA samples and assayed the expression of UGT2B17 cDNA using real-time PCR. We then assayed microsomes from these same human liver samples (HLMs) for activity against NNAL and SAHA using ultra-performance liquid chromatography (UPLC). We demonstrated that liver specimens from men exhibited an 8-fold higher expression of UGT2B17 mRNA than women (P-value < 0.001). In addition, HLMs from men exhibit a 2-fold higher glucuronidation activity against NNAL (P-value < 0.02) and a 2-fold higher glucuronidation activity against SAHA (P-value = 0.001). When stratifying this data by the genotype of the UGT2B17 gene deletion polymorphism, similar patterns were observed for glucuronidation of both substrates, with individuals with less than two copies of the UGT2B17 gene exhibiting less glucuronidation activity than individuals who have 2 copies of the UGT2B17 gene for both men and women. These data are consistent with a hormonal regulatory mechanism for UGT2B17 in humans and suggest that glucuronidation activities, at least for UGT2B17, may differ with gender. Women who smoke a comparable level of cigarettes have been shown to be at greater risk for lung cancer than men, this could be in part due to lower UGT2B17 expression and lower NNAL detoxification in women. This data may also have pharmacological implications in the dose of cancer therapy drugs (such as SAHA) given to men and women.

Human Embryonic Stem Cell Lines with Genetic and Chromosomal Disorders. *Y. Verlinsky, N. Strelchenko, V. Kukharenko, A. Sckumatov, S. Rechitsky, O. Verlinsky, A. Kuliev* Reproductive Genetic Inst, Chicago, IL.

Human embryonic stem cell (hESC) lines with abnormal genotypes provide an unlimited source for analysing the primary mechanisms of congenital disorders, and the development of the methods for cellular therapy. Based on our ongoing preimplantation genetic diagnosis (PGD) program, which currently includes more than 7000 PGD cases, we have developed the genetic disease specific hESC line repository, consisting of a total of 58 genetically abnormal lines. It contains eleven hESC lines with chromosomal abnormalities, including four translocations, two trisomies, two triploidies and three sex chromosomal aneuploidies, and 47 with different single gene disorders, thirteen obtained from the embryos with autosomal recessive, twelve X-linked and twenty two autosomal dominant disorders, the latter including hESC lines with dynamic mutations and those with genetic predisposition to cancer. The resulting collection represents the worlds first hESC line bank with genetic disorders, currently available for stem cell research of genetic disorders (www.stemride.com).

Combined Genome-wide Linkage and Association Analysis of extended Utah prostate cancer pedigrees identifies significance at 8q12. *GB. Christensen, J. Farnham, NJ. Camp, LA. Cannon-Albright* Dept Biomedical Informatics, University of Utah School of Medicine, Salt Lake City, UT.

We performed genome-wide linkage and case/control association studies in 27 prostate cancer cases from 2 extended, informative, high-risk Utah pedigrees. All relationships between cases within pedigrees were more distant than first degree. Genotyping was performed with the Illumina 550k SNP set, after exclusion of 58,000 markers failing quality control. For controls, we selected caucasians from the Illumina Icontrol data set (n=1,579), also genotyped for the 550k SNPs. Our initial screen for association included naive Fishers Exact Test, ignoring the familial relationships between cases, under three models: dominant, recessive, and an allele test. 54 distinct markers were selected for secondary screening with a significance cut off of p $1e^{-5}$. Secondary screening was performed using Genie software, which included known relationships between cases. In the secondary screen 1 marker reached the genome-wide significance threshold of p $3.4e^{-7}$. This marker was on chromosome arm 8q12 (p=$1e^{-7}$). In addition to providing the best overall GW association evidence, 5 of the top 8 associations from the secondary screening were also at 8q12; 9 SNPs in a 217kb region at chromosome 8q12 passed stage 1 screening. Other regions with markers reaching p $3e^{-6}$ included: 4 other markers at 8q12, 4p13, 2p25, 7p21, 17q22, and 21q21. We also performed linkage analysis in the 2 pedigrees. We selected a set of 27,157 SNPs from the Illumina 550k set, with no evidence of LD, and used the Smith (1996) inheritance model. Two regions showed suggestive evidence of linkage; chromosome arm 2p (hetLOD=2.44) and chromosome arm 8q12-q21 (max hetLod = 2.28). The SNPs showing significant evidence for association were in chr 8q12. This small study shows the power and synergistic utility of using both linkage and association analysis in high risk pedigrees. The 8q12 region identified as significant for prostate cancer predisposition has not been previously reported for linkage or association, but is recognized for LOH.

Dosage changes in both nuclear and mitochondrial genomes in autism spectrum disorders. *M. Smith, P. L. Flodman, C. Devine, M. A. Spence* Dept Pediatrics, Univ California, Irvine, Irvine, CA.

Lymphoblastoid DNA samples from 10 autism families, (8 triads and 2 with twins), were studied using the Affymetrix 6.0 SNP chip. Data were analyzed to identify in probands, de novo copy number changes in unique sequence DNA that did not overlap with known polymorphic copy number changes. The average number of such changes per proband was 11.5, range 1-58; roughly half were within genes. The number of variants was inversely correlated with proband IQ score. Genomic instability may be present in subject AU44-201 with 58 changes. He has a 559kb deletion on 18p11.2 that encompasses gene SMCDH1 (structural maintenance of chromosomes 1). Subject AU28-202 has 17 de novo unique sequence copy number changes. She is hemizygous for the NEIL1 gene, a glycosylase that repairs oxidation induced DNA damage. The variants found in our sample were not present in 30 triads in the HapMap data. The MZ twins in family AU 80 were identical for 99.8 percent of SNPs. However, they showed marked differences in the number of de novo unique sequence copy number changes; 14 in AU80-202 and 3 in AU80-203. Phenotypes differ markedly in the two, twin AU80-202 is much more severely affected. Three autism affected subjects from 3 different families showed duplication of a unique sequence segment on chromosome 19 that included genes GLTSCR2, EHD2, HBII 115 and SEPW. In subject AU44-201 the duplication extended further and included the GLTSCR1 gene. GLTSCR1 and 2 are brain expressed genes. We also identified unique sequence de novo copy changes within genes associated with autism in other studies. These genes included Glutamate receptor 6, (GRIK2) 6q16.3, CNTNAP2 on chromosome 7q35 and NLGN1 on 3q26.31. Variants in PCDH11X occurred in males in three families. It is possible that these variants may represent polymorphisms in PCDH11Y. Hybridization intensity was assessed for SNPs between 410 and 16,141 bases of the mitochondrial DNA sequence. In 8 of 11 autistic probands (3-18 years) there was reduced hybridization intensity within the 9.6-13.7 kb region, indicative of deletions of variable length. Decreased hybridization intensity in this region was also noted in 7 of 90 HapMap samples.

MLL PARTIAL TANDEM DUPLICATIONS ARE PRESENT IN CORD BLOOD SAMPLES. *D. Mercer[1], X. Hu[1], M. M. Li[1,2]* 1) Human Genetics Program, Tulane Univ Medical Sch, New Orleans, LA; 2) Department of Pediatrics; Tulane Univ Medical Sch, New Orleans, LA.

Leukemia-associated genetic alterations play important roles in leukemogenesis. They also serve as biological markers in the diagnosis, prognosis, treatment, and follow-up of hematopoietic malignancies. Specifically, partial tandem duplications (PTDs) of the MLL (mixed lineage leukemia) gene are strongly associated with AML (acute myeloid leukemia) and confer a poor prognosis to patients harboring them. We previously reported on the presence of MLL PTDs in the peripheral blood of healthy individuals and in prenatal samples when evaluated with nested RT-PCR. Upon confirming that these aberrations arise early in human development through cultured amniocytes and chorionic villi, we further sought to search for their presence in cord blood samples. We obtained 53 umbilical cord blood samples from newborns and performed nested RT-PCR to detect MLL PTDs. Forty of the 53 samples showed at least one MLL PTD (75%). Sequencing showed the most common exon fusions were 9/3 (20 samples), 10/3 (11 samples), and 11/3 (7 samples). In addition, we performed antibody staining and flow cytometry on a subset of cord blood samples and 2 known positive peripheral blood samples. Each of these blood samples were stained with CD2, CD7, and CD14 antibodies, then subjected to flow cytometry. Nested RT-PCR for MLL PTDs was repeated on each subfraction, which demonstrated presence of MLL PTDs in lymphoid subfractions and absence of MLL PTDs in myeloid subfractions. However, this may be due to poor myeloid cell recovery. The incidence of MLL PTDs in cord bloods was intermediate of that found in prenatal samples (100%) and peripheral bloods (49%), suggesting that the genetic abnormality occurs during cell division. Since MLL PTDs have been shown to be present throughout life in healthy individuals, other factors must be necessary to lead to malignant transformation. We suspect that one of these factors may be the cell lineage in which the alteration originates. A greater number of samples for flow cytometry are needed before this can be confirmed.

Motivators for participation in a whole genome sequencing study: The ClinSeq experience. *F. M. Facio, B. B. Biesecker, S. Brooks, J. Loewenstein, L. G. Biesecker* Natl Human Genome Res Inst, NIH, Bethesda, MD.

Introduction: ClinSeq is a pilot study to investigate and develop large-scale medical sequencing (LSMS) and whole genome sequencing (WGS) for clinical research. A distinctive aspect of ClinSeq is that subjects can choose to receive individual genotype results. Existing literature shows that altruism, benefits to self, and benefits to family are major motivators for research participation. ClinSeq provides a novel setting to examine motivators for participation in the context of LSMS/WGS. The purpose of this qualitative study was to explore the reasons individuals participate in a LSMS/WGS study. Methods: 337 individuals (age 45 to 65), who enrolled in ClinSeq between January 2007 and May 2008, were asked an open-ended question about their reasons for participating in the study and their demographics. Responses were imported into NVIVO 7 for coding and analysis. The sample size provided data saturation. The primary coder coded all 313 responses. The secondary coder coded 25% of the responses. Inter-coder reliability was 95.4%. Results: Of 337 enrollees, 313 (93%) provided responses. The majority were White (89%), highly educated (85%), and of high socio-economic status (66%). Two main themes were identified: altruism and seeking health information for oneself, with each theme arising from distinct groups. Conclusion: Our results show that ClinSeq subjects share motivations with both general research participants, as well as with those who come forth for genetic studies. Although personal health benefits is a more salient theme among disease cohorts than among healthy volunteers, many of our volunteers cited this as a motivator. Investigation of our subjects health related attributes will reveal how their background parallels this finding. To our knowledge this is the first cohort to undergo LSMS/WGS with the option of receiving individual genotype results, which provides a unique opportunity to study the theme of benefits to self in the context of personalized genomics research. We will discuss the implications of our results for future translational genomics research and describe follow-up studies to explore preferences towards LSMS/WGS.

Genome-wide Case Control Association Study for Familial Melanoma identifies 2 significant associations. *J. Farnham[1], SA. Leachman[2], NJ. Camp[1], LA. Cannon-Albright[1]* 1) Biomedical Informatics, University of Utah School of Medicine, Salt Lake City, UT; 2) Dermatology Department, University of Utah School of Medicine, Salt Lake City, UT.

We performed a genome-wide case control association study in 87 melanoma cases from 21 informative, high-risk Utah pedigrees. All relationships within pedigrees were more distant than first degree. Genotyping was performed with the Illumina 550k SNP set, after exclusion of 58,000 markers failing quality control and 14,000 markers on the X chromosome (not analyzed). For controls, we selected caucasians from the Illumina Icontrol data set (n=1,579); all were genotyped for the 550k SNPs. Our initial screen for association included naive Fishers Exact Test, ignoring the familial relationships between cases, under three models: dominant, recessive, and an allele test. 49 distinct markers were selected for secondary screening that surpassed a significance threshold of p $1e^{-5}$. Secondary screening was performed using Genie software, which accounts for the known relationships between cases. In the secondary screen 3 markers in 2 regions reached the genomewide significance threshold of p $3.4e^{-7}$. The most significant marker was on chromosome arm 6q22 ($p=5e^{-8}$). The 2 other markers were both on chromosome arm 16p13, located in A2BP1/FOX1 ($p = 2e^{-7}$ for each). A2BP1, ataxin-2 binding protein-1 (A2BP1) gene, also called FOX1. Other regions with markers reaching p $5e^{-6}$ included: chromosome arms 2q12, 2q24, 2q32, 8p23, 12p13, 17p11, 18q22, and 20q13 (20Mb from the recently reported association at 20q11). This study represents the first complete genome wide association study reported for familial melanoma. This study shows the power and utility of association analysis in related cases from high-risk pedigrees. Two significant regions of association were identified, neither of which has been previously reported.

Genetic Admixture, Preterm Delivery and Related Traits among African American Mothers. *H. J. Tsai[1, 2], Y. Yu[1, 2], S. Zhang[1, 2], C. Pearson[3], K. Ortiz[3], X. Xu[4], H. Bauchner[3], B. Zuckerman[3], X. Wang[1, 2]* 1) Mary Ann and J. Milburn Smith Child Health Research Program, Childrens Memorial Hospital and Children's Memorial Research Center, Chicago, IL; 2) Dept Pediatrics, Northwestern Univ Sch Med, Chicago, IL; 3) Department of Pediatrics, Boston University School of Medicine and Boston Medical Center, Boston, MA; 4) Center for Population Genetics, University of Illinois at Chicago School of Public Health, Chicago, IL.

In the U.S., the rate of preterm delivery (PTD) is significantly higher in African Americans (17.8%) than non-Hispanic whites (11.5%). Such disparity cannot be fully explained by differences in socio-environmental factors. In this study, we used 57 ancestry informative markers (AIMs) to estimate genetic ancestry in 812 mothers enrolled in a case-control PTD study at Boston Medical Center who self-reported their ethnicity as black. We first estimated ancestral proportion among African American mothers using AIMs. Second, we investigated the association of genetic ancestry with PTD and related traits. Finally, we examined the potential population stratification confounding in this case-control PTD study. The estimated average African ancestral proportion was 0.900.13 in this sample. We found significant associations of ancestral proportion with PTD as a whole and PTD subgrouped by the presence of maternal hypertensive disorders. In addition, marginal associations were found with spontaneous PTD, medically induced PTD and very PTD. We did not observe significant confounding due to population stratification in this case-control PTD study. In summary, African ancestral background was significantly associated with an increased risk of PTD as a whole and within certain preterm subgroups. Our data underscore the need for more intensive investigation of genetic admixture in African Americans such as genome-wide admixture mapping to identify novel susceptibility genes of PTD.

Kernel Based Adaptive Cluster (KBAC): A Powerful Method to Detect Associations for Complex Traits due to Rare Variants in the Presence of Gene x Gene and Gene x Environment Interactions. *D. J. Liu[1,2], S. M. Leal[2]* 1) Department of Statistics, Rice University, Houston, TX 77005; 2) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX 77025.

Common diseases can be due to multiple functional variants with allele frequencies ranging from rare to common. Both gene x gene and genes x environment interactions can play a role in disease susceptibility. Although whole genome association studies using tagSNPs are a powerful approach for detecting common variants, they are underpowered to detect associations with rare variants due to indirect mapping. The development of cost-effective sequencing technologies enables the detection of rare variants for use in association studies. Although methods used for the analysis of common variants are applicable to sequence data, their performance is poor for analyzing rare variants and their power may be further reduced in the presence of interactions. We developed a novel method, *Kernel Based Adaptive Cluster* (KBAC) to carry out direct association studies of rare variant data. This method can be used for gene mapping with or without the presence of gene x gene and gene x environmental interactions. Significance for the KBAC method can either be determined empirically through permutation or using the derived asymptotic distribution when the sample size is sufficiently large. It is demonstrated through extensive simulations motivated by real data (e.g. Hirschsprungs Disease, Breast Cancer) that the KBAC method has superior performance than univariate and multivariate tests for detecting associations with or without interactions. The KBAC method is powerful and robust even in the presence of misclassification error where either non-causal variants are included in or casual variants are excluded from the analysis. The KBAC method can be applied to the analysis of sequence data from either whole genomes or candidate genes.

Harnessing Nature's Powerful DNA Sequencing Engine: Single Molecule Real Time Sequencing-by-Synthesis. *S. W. Turner* Pacific Biosciences, 1505 Adams Drive, Menlo Park, CA.

SMRT (single molecule real time) DNA sequencing is a novel, high throughput method for sequencing DNA. Historically, the majority of DNA sequence data collected has been acquired through the use of DNA polymerase enzymes. However, the methods used squander the inherent power of the enzyme as a sequencing engine. Viewed as such, DNA polymerases can read up to 1000 bases per second per molecule, do so over DNA lengths of 100,000 bases or more, replicate with high fidelity and consume only one molecule per base sequenced. To harness this power, Pacific Biosciences has developed a method of eavesdropping on template-directed synthesis by DNA polymerase in real-time. Two critical technology components enable this process: The first is phospholinked nucleotides where, in contrast to other sequencing approaches, the fluorescent label is attached to the terminal phosphate rather than the base. The enzyme cleaves away the fluorophore as part of the incorporation process, leaving behind completely natural double-stranded DNA. The second critical component is zero-mode waveguide (ZMW) confinement technology that allows single-molecule detection at concentrations of labeled analogs relevant to the enzyme. Through the combination of these innovations, our technology allows the speed, processivity, efficiency and fidelity of the enzyme to be exploited. We show proof-of-concept data that indicate this will be a high-throughput sequencing technology. We will present a novel sample prep concept that facilitates whole genome shotgun sequencing directly from genomic DNA, compatible with molecular consensus sequencing that enables detection of rare mutants. We will also show how the wealth of biophysical data that emerges from real-time sequencing enables biological and medical studies previously inaccessible to sequencing technology.

A network-based analysis of GWAS in multiple sclerosis identifies immunological and neural pathways associated with disease susceptibility. *S. E. Baranzini[1], M. R. Barnes[2], J. Wang[1], R. Lindberg[4], P. Khankhanian[1], P. M. Matthews[2], L. Kappos[4], C. Polman[3], S. L. Hauser[1], R. A. Gibson[2], J. R. Oksenberg[1], N. W. Galwey[2]* 1) Department of Neurology, UCSF, San Francisco, CA; 2) GlaxoSmithKline Research and Development, Harlow, England; 3) Department of Neurology, Vrije Universiteit Medical Center, Amsterdam, Netherlands; 4) Neurology and Department of Biomedicine, University Hospital Basel, Basel, Switzerland.

Multiple sclerosis (MS) is a common and severe neurological disease of young adults. Genome-wide association studies (GWAS) testing several hundred thousand SNPs have been performed in MS and other complex diseases. Typically, the number of markers in which the evidence for association exceeds the genome-wide significance threshold is very small, and markers that do not exceed this threshold are generally neglected. Classical statistical analysis of these datasets in MS revealed genes with known immunological functions. However, many of the markers showing modest association may represent false negatives. We hypothesize that certain combinations of genes harboring these types of markers can be identified if they belong to a common biological pathway. Here we conduct a pathway-oriented analysis of two GWAS in MS that takes into account all SNPs with nominal evidence of association ($p<0.05$). Gene-wise p-values were superimposed on a human protein interaction network and searches were conducted to identify sub-networks containing a higher proportion of genes associated with MS than expected by chance. These sub-networks, and others generated at random as a control, were categorized for membership of biological pathways. GWAS from 8 other diseases were analyzed to assess the specificity of the pathways identified. In the MS datasets we identified sub-networks of genes from several immunological pathways including cell adhesion, communication, and signaling pathways. Remarkably, neural pathways, namely axon-guidance and synaptic potentiation, were also over-represented in the MS datasets. In conclusion, in addition to the immunological pathways previously identified, we report here for the first time the potential involvement of neural pathways in MS susceptibility.

Lower *IL2RA* Expression by Cis-acting Regulation Increases T1D Genetic Susceptibility. *H. Q. Qu[1], D. J. Verlaan[2], B. Ge[2], Y. Lu[1], C. L. K. Lam[2], L. Marchand[1], E. Harmsen[2], T. J. Hudson[3], T. Pastinen[2], C. Polychronakos[1]*
1) Endocrine Genetics, McGill University Health Centre, Montreal, Québec, Canada; 2) McGill University and Genome Québec Innovation Centre, Montréal, Québec, Canada; 3) Ontario Institute for Cancer Research, Toronto, Ontario, Canada.

Background The expression of *IL2RA* is an important marker of $CD4^+CD25^+$ regulatory T cells (T_{reg}). Targeting at *IL2RA*, IL2 treatment to promote the expansion of T_{reg} is in clinical trials for autoimmune diseases (ClinicalTrials.gov NCT00525889). Association of T1D with the *IL2RA* locus was first identified using a multilocus test(Vella,et al.2005). This association was replicated independently in our dataset of 949 European family trios, and mapped to the 5' of the *IL2RA* region(Qu,et al.2007). A recent study reported that the most significant T1D association was with -10,388 C/A (ss52580101, Lowe,et al.2007). **Objectives** (1)To see whether ss52580101 entirely accounts for the T1D association; (2)To detect and map cis-regulatory variation of *IL2RA* levels with a potential to explain the T1D association. **Methods** We measured differential allelic expression in unspliced heteronuclear RNA by normalized sequencing (Ge, et al. 2005, Pastinen, et al. 2005) in 55 parental LCLs of CEU (the European HapMap set) and 18 parents of diabetic children at four intronic SNPs. Differences in allele ratios between DNA and the corresponding RNA were taken as evidence of a transcriptional effect and correlated to genotype at T1D-associated makers. **Results** (1)The T1D association could not be entirely explained by ss52580101 as the effect of a SNP in intron 1 remained significant ($P=7.15x10^{-4}$) after regression for ss52580101. (2)We identified a haplotype, marked by the T1D predisposing allele of rs3118470, which is highly associated ($P=5.58x10^{-6}$) with a transcriptional effect resulting in lower *IL2RA* mRNA level. **Conclusion** This transcriptional allelic effect provides a plausible explanation of the T1D susceptibility: lower *IL2RA* expression may decrease IL2 signaling in the suppression of autoimmunity by T_{reg}, consistent with a similar effect of lower IL2 production determined by the *Idd3* locus in the NOD mouse(Yamanouchi, et al. 2007).

A multistage genome-wide SNP association study identifies a novel gene associated with susceptibility to type 2 diabetes mellitus. *K. Miyake[1], K. Yasuda[2], Y. Horikawa[3], M. Kasuga[1,2], Study Group of the Millennium Genome Project for Diabetes Mellitus* 1) Kobe University Graduate School of Medicine, Kobe, Japan; 2) Research Institute, International Medical Center of Japan, Tokyo, Japan; 3) Gifu University School of Medicine, Gifu, Japan.

As part of a national project designated the Millennium Genome Project in Japan, we performed a multistage genome-wide association study (GWAS) of type 2 diabetes mellitus (T2D) in Japanese with 100,000 SNPs. In the first stage, we compared the 187 diabetic subjects with two different control groups, reference data for 752 subjects in JSNP database and the 752 subjects for the other disease groups of this project. Two additional rounds of screening (second stage: cases vs. controls = 752 vs. 752, third stage: 672 vs. 672) identified 10 SNPs associated with T2D in the Japanese population. The most significant association was obtained with Gene-A, which had not been reported by GWASs in Caucasians, and dense mapping within the gene revealed that a common SNP showed the lowest *P* value of 6.7×10^{-13} (odds ratio (OR) = 1.49). In two independent Japanese panels (1521 vs. 1544 and 1433 vs. 1444), this SNP was reproducibly associated with the risk of T2D ($P = 9.6 \times 10^{-10}$ and 6.9 × 10-^{10}). The meta-analysis with 8790 Japanese subjects yielded a *P* value of 3.0×10^{-29} (OR = 1.43). Replication studies for the association of this gene with T2D in other Asian population as well as Caucasian are under way. Among control subjects, the risk allele of this SNP was associated with impairment of insulin secretion. Since none of the T2D susceptibility genes, which were previously revealed by GWASs in Caucasians, were detected in this multi-stage GWAS, we performed replication studies using the Japanese panels for eleven loci. We found that *TCF7L2*, *SLC30A8*, *HHEX*, *CDKN2B*, *IGF2BP2* and *CDKAL1* were reproducibly associated with T2D in Japanese. In summary, we identified a novel T2D susceptibility gene by a multistage genome-wide association study and also found the reproducibility of six T2D susceptibility genes in Japanese.

Orthotopic Liver Transplant for Arginase Deficiency. *B. Shaffer[1], S. Shankar[1], E. Nicholas[1], K. Weisiger[1], C. Zlatunich[1], W. O'Brien[2], S. M. Kang[3], L. Ferrell[4], P. Rosenthal[5], S. Packman[1]* 1) Medical Genetics Dept, UCSF, San Francisco, CA; 2) Molecular and Human Genetics Dept, BCM, Houston, TX; 3) Surgery Dept, UCSF, San Francisco, CA; 4) Pathology Dept, UCSF, San Francisco, CA; 5) Pediatric Gastroenterology, UCSF, San Francisco, CA.

Individuals with arginase deficiency (Arg Def) often suffer from poor growth, plateaued cognitive development, neurodevelopmental decline, spastic diplegia, loss of ambulation, and loss of bladder and bowel function. These manifestations occur despite few episodes of significant hyperammonemia, and, in the face of adherence to medical and dietary management. Orthotopic liver transplantation (LT) has not been employed in treatment of Arg Def. We describe the clinical course of one such case. Our patient is a 30 mo boy identified through newborn screening. Arg Def was confirmed by red blood cell enzyme assay that showed *zero* activity. Treatment with with Buphenyl and reduced arginine intake was begun. He had several mild hyperammonemic episodes with rapid resolution on treatment. Liver transaminases were intermittently elevated (AST/ALT up to 10X nl) without evidence of liver failure; abdominal ultrasound was normal. However, linear growth fell from the 75th centile at 10 mo to <5th at 29 mo; weight fell from the 90th to the 50th centile. Arginine levels (checked biweekly) were typically 2X nl (~50% of values), but were as high as <5X nl (~5% of values). Gastrostomy tube was placed at 27 mo to facilitate nutritional management. Spastic diplegia was noted at 26 mo, with progressively worsening contractures and decline in verbal skills by 28 mo. To prevent further deterioration, he underwent an uncomplicated LT at 29 months. Histologic examination of the explant revealed areas of pale swollen cells in peri-portal to mid zonal location. Post-transplant arginine levels normalized rapidly on a normal diet. His neurologic examination revealed reductions in spasticity and reflexes. With due recognition of the risks of LT, we submit that in Arg def with early neurologic or cognitive decompensation, LT is warranted, to potentially alter the disease impact and natural history of this disorder.

Sequence analysis of small RNAs present in preimplantation mouse embryos. *Y. Ohnishi[1,2], A. Toyoda[3], Y. Totoki[3], T. Watanabe[4], H. Sasaki[4], K. Tokunaga[2], Y. Sakaki[3], H. Hohjoh[1]* 1) National Institute of Neuroscience, NCNP, Tokyo, Japan; 2) Department of Human Genetics, Graduate School of Medicine, Univ Tokyo, Tokyo, Japan; 3) RIKEN Yokohama Institute, Yokohama, Japan; 4) Division of Human Genetics, Department of Integrated Genetics, NIG, Mishima, Japan.

Small RNAs (18-32 nucleotides in length) including small interfering RNAs (siRNAs) and microRNAs (miRNAs) are thought to play an essential role in biological functions. In this study, we focused on the small RNAs present in the course of early development of mouse embryo and constructed cDNA libraries for such small RNAs at the stages of oocyte, compacted 8- to 16-cell embryo (2.5 d.p.c.) and blastocyst (3.5 d.p.c.). After sequencing and annotation of clones, we found that the small RNAs derived from retrotransposon and miRNAs were abundantly present in oocyte and blastocyst, respectively. From the sequence analysis, most of the miRNAs (e.g., miR-290 cluster) appeared to be expressed in the 8-cell stage embryo and blastocyst. In contrast, maternally derived miRNAs (e.g., miR-let-7 family) seemed to be decreased during early development. The difference in the miRNA expression levels were also confirmed by RT-real time PCR. We further examined the small RNAs derived from retrotransposons and pseudogenes in oocytes and embryos, since recent studies showed that endogenous siRNAs generated from retrotransposons and pseudogenes were able to regulate their own expression by RNAi in growing oocytes. [Watanabe et al. Nature, 453(7194): 539-43, Tam et al. Nature, 453(7194): 534-8]. Of such candidates, we focused on small RNAs derived from LINE-1 (L1) retrotransposon, which were abundantly present in preimplantation embryos. When the GFP transcripts carrying the L1 sequence in its 3-UTR was introduced into fertilized egg, it was observed that the transcript was significantly decreased in 2-cell stage embryo. In addition, the level of L1 appeared to be increased in *Dicer* knock down embryos. Taken together, the results suggest that small RNAs derived from the L1 retrotransposon may be also involved in regulation of its expression in preimplantation mouse embryos.

Physician's attitude for New Genetic Testing services in Japan. *T. Ohata, A. Tsuchiya, M. Watanabe, T. Sumida, F. Takada* Dept Med Gen,Grad Sch Med Sci, Kitasato Univ, Sagamihara, Japan.

The purpose of genetic testing, users of genetic testing, and places to be provided genetic testing have been diversifying in Japan. Some New genetic testing for predicting disease susceptibility of multifactorial diseases have been appeared, and are provided by some clinics not specialized in medical genetics or sold to consumers via web sites directly. We conducted the survey of physicians (7528 general practitioners: GPs and 503 clinical geneticists: CGs) about new genetic testing services in 2007. The clinical validity and utility of new genetic testings that are actually provided were evaluated by those physicians.

Percentage of physicians who answered "useful "

	GPs	CGs
Obesity	55.8	31.4
Hypertension	62.2	37.0
Diabetes Mellitus	65.4	33.1
Alzheimer disease	58.4	35.7

General practitioners considered these testing more useful compared to clinical geneticists. These findings suggested that these new genetic testing services may be provided by physicians not specialized in medical genetics. Considering the possibility of expanding the use of genetic testing, it is necessary to establish integrative criteria to assess the analytical and clinical validities and clinical utility of genetic testing, share information necessary to provide testing, such as counseling information.

The effect of blood glucose variability on the development and progression of microvascular complications: results from the DCCT/EDIC study on type I diabetes mellitus (DM). *A. S. Rigby[1], S. L. Atkin[2], E. S. Kilpatrick[3]* 1) Academic Cardiology, University of Hull, UK; 2) Department of Diabetes, HYMS, UK; 3) Department of Clinical Biochemistry, Hull Royal Infirmary, UK.

The risk of developing microvascular complications in DM is related to the glycemic control of an individual. What remains controversial is whether glycemic instability may confer a risk to complications in addition to that predicted by the mean blood glucose (BG) value alone. We wanted to determine whether glucose variability during the original period of the DCCT influenced long-term risk of developing microvascular complications during EDIC. In the DCCT a capillary BG profile and HbA1c was taken quarterly. BG was assessed at 7-points daily: pre/post breakfast, pre/post lunch, pre/post supper & bedtime. Mean BG was calculated as an area under the curve. Instability of BG (within-day) during the DCCT was calculated using 2 methods: the standard deviation (SD) of daily blood glucose around the mean from each quarterly visit; also as the mean amplitude of glycemic excursion (MAGE). HbA1c measurement (unlike that of glucose profiling) continued after the DCCT into EDIC. Retinopathy development/progression was defined as a change of >=3 units in the 25-point Early Diabetic Retinopathy Treatment Study score since the end of the DCCT. Analyses were stratified by DCCT treatment group (intensive/conventional). GEE logistic regression was used to assess the effect of glycemic variables on retinopathy over repeated time-points adjusting for age, sex and disease duration. The number (%) of patients with retinopathy at years 1, 2, 3 and 4 of EDIC was 15/369 (4%), 37/443 (8%), 47/419 (11%) and 146/1208 (12%) respectively. In the intensive group there was no significant relationship between BG variability and retinopathy (SD: OR=0.92, 95% CI= 0.72,1.15, P=0.45; MAGE: OR=0.99, 95% CI=0.88,1.10, p=0.81). Both mean BG during the DCCT (OR=1.29, 95% CI=1.16,1.43, p<0.0001) and mean HbA1c in EDIC (OR=1.35, 95% CI =1.14,1.60, p=0.001) were significantly associated with retinopathy. It is reassuring that patients with large glycemic variability are at no greater risk to retinopathy than their mean BG level suggests.

Infantile-onset ascending spastic paralysis (IAHSP) caused by maternal uniparental heterodisomy with partial isodisomy of a chromosome 2 harboring a novel splice acceptor site mutation (IVS9-2AT) in the gene *ALS2*. *U. Muller[1], N. Wolf[2], P. Winter[1], H. Hackstein[3], D. Vater[2], T. Herzfeld[1]* 1) Inst. of Human Genetics, Justus-Liebig Univ, Giessen, Germany; 2) Dept. of Child Neurology, Ruprecht-Karls-Universität, Heidelberg, Germany; 3) Inst. of Clinical Immunology and Transfusion Medicine, Giessen, Germany.

Infantile-onset ascending spastic paralysis (IAHSP, OMIM #607225) is a rare autosomal recessive early onset motor-neuron disease. Mutations in the gene *ALS2* are the underlying cause of IAHSP and of the two clinicallly related motor neuron diseases juvenile primary lateral sclerosis (JPLS, OMIM #606353) and juvenile amyotrophic lateral sclerosis (ALS2, OMIM #205100). To date 14 mutations have been described in *ALS2*. Thirteen are homozygous and one is compound heterozygous. Most mutations (9) were detected in patients with IAHSP. We analyzed *ALS2* in a German IAHSP patient with non-consanguineous parents. Sequencing of all 34 exons and intron/exon boundaries of *ALS2* identified a homozygous splice acceptor site mutation in intron 9 of *ALS2* (IVS9-2AT). RT-PCR experiments showed that the mutation results in skipping of exon 10. This causes a frame-shift in exon 11 and a premature stop codon. We then investigated the parents and found the IVS9-2AT mutation in the heterozygous state in the mother but not in the father. Once non-paternity was excluded, we analyzed various polymorphic loci on chromosome 2 in the parents and in the patient. The absence of paternal alleles indicated maternal uniparental disomy. While homozygosity was observed at several loci including *ALS2*, other loci were heterozygous, i.e. both maternal alleles were present. The findings demonstrate maternal uniparental heterodisomy with partial isodisomy of chromosome 2 in the patient. A likely mechanism is non-disjunction during meiosis I and subsequent trisomy 2 rescue or gamete complementation. This case emphasizes that non-consanguineous parents of children homozygous for a rare autosomal recessive disease should to be tested before giving risk estimates for subsequent children.

Pontine dysplasia in a child with CHARGE syndrome. *J. van den Ende[1], K. Plaskie[2], P. Govaert[3]* 1) Dept Medical Genetics, University Hospital Antwerp, Wilrijk, Belgium; 2) Neonatal Intensive Care Unit, University Hospital Antwerp, Wilrijk, Belgium; 3) Neonatal Intensive Care Unit, Sophia Childrens`s Hospital- Erasmus MC, Rotterdam, the Netherlands.

CHARGE syndrome is an autosomal dominant condition caused by mutations in the CHD7 gene. The clinical picture is quite variable, between as well as within families. There does not seem to be a clear genotype-phenotype correlation. Before the gene was identified clinical criteria were used to make the diagnosis. The most important criteria were: coloboma, heart defects, choanal atresia, retarded growth/ development, genital hypoplasia, and ear anomalies/deafness, making up the acronym CHARGE. Now that the clinical picture is becoming more defined other features seem to be more important, like for example orofacial clefts and CNS malformations. Hypoplasia of the semi-circular canals and defects in the olfactory bulb development appear to be present in virtually all patients with CHD7 mutations. This finding, together with the fact that even subtle CNS malformations in CHARGE syndrome are associated with increased mortality, stresses the importance of neuro imaging, preferably by MRI, in all cases with even mild suspicion of CHARGE syndrome. We describe a case of a newborn with cleft lip and palate, deafness, and severe breathing problems, lacking most of the primary criteria for CHARGE syndrome. Subtle alterations in the pontine/cerebellar region on the ultrasound and MRI scan of the brain raised the suspicion of CHARGE syndrome. Subsequently a CT scan of the middle ear revealed absence of normal semi-circular canals, and finally DNA analysis of the CHD7 gene confirmed the diagnosis of CHARGE syndrome.

Genetic causes for inner ear malformation (Mondini Dysplasia, Enlarged Vestibular Aqueduct- and Pendred Syndrome). *R. Birkenhager, R. Laszig, A. Aschendorff* Department of Otorhinolaryngology, Head and Neck Surgery, University Hospital, Killianstrasse 5, D-79106 Freiburg, Germany.

Background: Pendred-Syndrome, an autosomal-recessive disorder is characterized by sensorineural deafness and goiter. This syndrome is one of the most common forms of syndromic deafness. Hearing loss is prelingual in the majority of the cases, only a subset of patients have a progressive hearing loss later in life. The deafness is associated with temporal bone abnormalities ranging from isolated enlargement of the vestibular aqueduct to Mondini dysplasia, a more complex malformation that also includes cochlear hypoplasia. PDS is caused by mutations in the SLC26A4 gene. The SLC26A4 gene is expressed in the non-sensory epithelia of the inner ear. Recently an additional gene FOXI1 was described which mayalso be responsible for Pendred- Syndrome and Mondini dysplasia. The FOXI1 gene is involved in the transcriptional control of the SLC26A4 gene. In this study we analysed 64 patients with EVA and hearing loss to distinguish between Pendred-, EVA-syndrome and Mondini dysplasia. **Methods:** Individual exon and intron transitions of the SLC26A4 and FOXI1 gene of patients were PCR sequenced. A genome-wide linkage analysis was accomplished using the 500 K genotyping. **Results:** In the analysed patients with Pendred-Syndrome and/or enlargement of the vestibular aqueduct, a total of twenty-one different SCL26A4 mutations were detected, by contrast mutations could not be detected in the FOXI1 gene. In the SLC26A4 gene a mutation could not be detected in 42 % of the cases. With a genomewide SNP analysis, of these families it was possible to identify potential new gene loci responsible for the Enlarged Vestibular Aqueduct Syndrome and Mondini dysplasia. **Conclusions:** These results suggested in contrast to the literature that the FOXI1 gene is not the main factor involved in Pendred- and Enlarged Vestibular Aqueduct Syndrome. Our results indicate evidences of an accessory gene for the Enlarged Vestibular Aqueduct Syndrome and specially for Mondini dysplasia.

DNA integrity and seminal antioxidant analysis in idiopathic infertile Indian men. *M. B. Shamsi[1], S. Venkatesh[1], P. Talwar[2], R. K. Sharma[2], S. Mukherjee[3], R. Kumar[3], S. Arora[4], D. S. Arya[4], R. Dada[1]* 1) Dept. of Anatomy, AIIMS, New Delhi, India; 2) ART Centre.Army Research and Referral Hospital New Delhi, India; 3) Dept of Urology, AIIMS, New Delhi, India; 4) Department of Pharmacology. AIIMS, New Delhi India.

Infertility affects about 15 -20 % people in the reproductive age. About 30 - 40 % of infertile men have raised Reactive Oxygen Species levels and low antioxidant levels, leading to oxidation of nucleotides and malondialdehyde production from membranes by lipid peroxidation. Seminal plasma from 39 idiopathic infertile males and 27 healthy controls was evaluated for Superoxide Dismutase (SOD), Glutathione (GSH), Malondialdehyde (MDA) and Catalase (Cat) by biochemical method. The DNA integrity of sperms was assessed by Single Cell Gel Electrophoresis (SCGE). The Comets in SCGE were classified into four categories (category A- no DNA migration to D-highest DNA migration) as observed by a micrometer attached to the eyepiece.Significant difference (p=0.492) in the assayed AO and MDA levels were observed in infertile patients and healthy controls. Motility was positively correlated with AO and negatively with MDA levels in both the patient and control group. MDA had positive correlation (p=0.471) with sperm morphological deformities. No correlation between sperm concentration and AO was observed in study group but an increased MDA level was associated with sperm count in patients but not in controls. Greater group D comet images were observed in infertile patients having low seminal antioxidant or higher MDA profile. The viability was positively associated with the number of comets in category A in patients and controls. Greater the sperm number higher will be the MDA production in OS, therefore in patient group a direct correlation of MDA and sperm number was observed whereas in controls due to insignificant OS no such correlation was observed. OS induces DNA aberrations, leading to apoptosis in the sperm leading to longer tails in the SCGE and decreased viability of sperms. This study provides an insight to the important role played by AO and DNA integrity in the maintenance of reproductive potential of sperms.

Rare de novo CNVs: Matching mouse gene knockout models to human mental retardation. *C. Webber[1], J. Y. Hehir-Kwa[2], DQ. Nguyen[1], B. B. A. de Vries[2], J. A. Veltman[2], C. P. Ponting[1]* 1) MRC Functional Genomics Unit, Oxford University, Oxford, United Kingdom; 2) Department of Human Genetics, Nijmegen Centre for Molecular Life Sciences, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands.

Rare structural genomic variations such as copy number variants (CNVs) frequently underlie common neurological disorders including mental retardation (learning disability), autism and schizophrenia. A major impediment to the clinical interpretation of these variants is that CNVs are also widespread in the general population. We have analysed a set of 148 rare genomic structural variants associated with mental retardation that were gathered from 17 publications and the DECIPHER database. In addition to Gene Ontology, tissue-expression and functional pathway annotations, we exploited the new wealth of genomic data from over 4000 mouse gene knockout experiments to forge links between these structural variants and their associated phenotypes. Mental retardation-associated structural variants were significantly enriched in (i) genes whose mouse orthologues, when disrupted, result in specific neuronal phenotypes, (ii) genes with known roles in human neurodegenerative pathways, in particular Parkinsons disease (iii) genes with enhanced expression in the brain relative to other tissues, and (iv) genes involved in transcriptional regulation. We find a significant association between the phenotypes from mouse gene knockout experiments and additional phenotypes of individual patients, such as cleft palate. Furthermore, we find that most of these enrichments were increased after removing those genes also overlapped by apparently benign CNVs. By exploiting these enrichments we have identified 125 genes enriched in variants that contribute to mental retardation and associated phenotypes.

Kluver-Bucy syndrome In a Child with Neurofibromatosis - type 1 (NF-1). *M. Miller[1], L. Kleiner[2]* 1) Dept Medical Gen, Children's Med Ctr, Dayton, OH; 2) Dept Neurosurgery, Children's Med Ctr, Dayton, OH.

Kluver-Bucy syndrome is a very rare, human behavior disorder that was originally described in monkeys who had both temporal lobes removed. These monkeys had visual agnosia, altered sexual behavior, and oral stimulation behaviors. Humans who incur bilateral temporal lobe dysfunction can also develop a similar clinical picture as in the temporal lobe lobectomized monkeys, but they do not typically show all of the clinical features. Kluver-Bucy syndrome has been described in humans in the following clinical settings: herpes simplex encephalitis (most common etiology), head trauma, severe dementia, Pick's disease, surgical lesions, and cerebrovascular disease. It is very rare in children, and has not been described in NF-1. AH is a 10 year old male with known NF-1 based on multiple cafe-au-lait spots and a prior optic glioma. His elementary school principal sent a letter to his treating physicians pleading for help in trying to understand his bizarre and threatening behaviors including: hypersexualism (he is constantly touching boys and girls in their genitals and grabbing girls breasts), inappropriate emotional responses (he would giggle and smile when being disciplined), and outbursts of anger. Review of his head MRI showed that on T2 sequence there were bilateral and symmetrical 1.3 cm lesions of high signal intensity (UBOs= unidentified bright objects) in the amygdala of both temporal lobes, findings thought to be consistent with Kluver-Bucy syndrome. This is the first example of Kluver-Bucy syndrome in association NF-1 in which there are the typical UBOs of NF-1 located in the anatomic region associated with Kluver-Bucy syndrome, the medial temporal lobes.

Model-independent linkage analysis and tests of association for familial idiopathic scoliosis and a candidate region on chromosome 6. *C. Justice[1], N. H. Miller[2], B. Marosy[3], D. Behneman[1], A. F. Wilson[1]* 1) Genometrics Section, IDRB, National Human Genome Research Institute, NIH, Baltimore, MD; 2) University of Colorado, The Childrens Hospital, Denver; 3) Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD.

Familial idiopathic scoliosis (FIS) is a lateral curvature of the spine present in the late juvenile or adolescent period in otherwise normal individuals. It affects 2-3% of the pediatric population, and 0.2-0.5% of the population require active treatment. Idiopathic scoliosis is believed to be a complex genetic disorder in which expression of the disease state may depend on several genetic and possibly environmental factors. Previous studies have suggested autosomal dominant, X-linked and multifactorial modes of inheritance. As part of a large collaborative study of FIS, 202 families with at least two individuals with a lateral curvature greater than or equal to 10 degrees were ascertained and clinically characterized. A genomic-wide screen identified candidate regions on chromosomes 6, 9, 16 and 17 [Miller et al. 2005]. The candidate region on chromosome 6 was genotyped with SNPs using the Ilumina platform. SNP marker density was ~ 58 Kb, with 537 SNPs genotyped on 6q13-q21. FIS was analyzed both as a quantitative and a qualitative trait, in which the curvature determining the threshold for affectation status was set at values of 10 and 30 degrees. Model independent sib-pair linkage analysis was performed with SIBPAL [S.A.G.E., v5.0, Case Western Reserve University, Cleveland, OH]. Tests of association were performed with FBAT [Rabinowitz and Laird 2000; Laird, Horvath, and Xu 2000; Horvath et al. 2004] for FIS as a qualitative trait and with ASSOC [S.A.G.E., v5.0] for FIS as a quantitative trait. Haplotypes of two, three and four SNPs were also tested for association with FBAT. The most significant results for the linkage analysis were obtained when the affectation status was set at 10 degrees or greater, with p-values 0.05 in a region spanning from 74 to 80 Mb. Association analyses for the whole sample resulted in several significant p-values.

NR4A2: Modeling Cellular Lithium Response. *R. C. McEachin[1,2], H. Chen[1], B. J. Keller[3,2], A. R. Prossin[1], Y. Bai[2], N. E. Carlson[4], P. Zandi[5], M. G. McInnis[1,2]* 1) Dept Psychiatry, Univ Michigan, Ann Arbor, MI; 2) National Center for Integrative Biomedical Informatics, Univ Michigan, Ann Arbor, MI; 3) Dept Computer Science, Eastern Michigan University, Ypsilanti, MI; 4) Dept Biostatistics, University of Colorado, Denver, CO; 5) Dept Mental Health, The Johns Hopkins University, Baltimore, MD.

Lithium is effective in the treatment of mania for approximately 70% of patients with Bipolar Disorder (BD). However, the mechanism of lithium's action is poorly understood. Using a new algorithm called Prioritizing Disease Genes by Analysis of Common Elements (PDG-ACE), we developed a novel, statistically significant, biologically plausible hypothesis on the genetic etiology of lithium's action in treating bipolar mania. Since lithium exerts an environmental influence on cells, we first identified candidate genes differentially expressed with lithium treatment in a cellular model, starting with Illumina's Human Ref8_V2 BeadChip and confirming these results by RT-PCR. Based on these analyses, we prioritized NR4A2 and FOS for further analysis and applied the PDG-ACE algorithm. PDG-ACE results led us to hypothesize that NR4A2 and FOS modulate cellular responses to lithium via dopamine signaling. Using GeneGo's MetaCore database, we developed a genetic model incorporating the hypothesized influences of lithium on dopamine signaling via NR4A2 and its network of interactors. Genes in this network are significantly enriched (p-value < 0.01) for "lithium", "dopamine", and "Bipolar Disorder" in MeSH annotation of publications tagged for these genes. In addition, 13 of the 50 genes in this network are under NPL interactions peaks for BD (p-value < 0.01). The hypothesis developed and tested in this analysis is consistent with a role for the NR4A2 network in cellular responses to lithium treatment, models response and non-response of individuals to lithium treatment, and is consistent with comorbid substance abuse often seen in BD. We also propose novel candidate genes for understanding lithium's action in BD, including genes that are known therapeutic targets of other drugs that may be useful in treatment of BD.

Identification of novel *HMGA1* and *HMGA2* fusion genes in lipoma. *X. Wang, R. L. Hulshizer, R. Q. Zamolyi, V. Pannain, M. R. Johnson, R. B. Jenkins, R. V. Lloyd, A. M. Oliveira* Dept of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN.

Lipomas are among the most common benign soft tissue tumors. Cytogenetic and molecular genetic analyses have shown that 60-80% of them contain rearrangements of the chromatic remodeling genes *HMGA2* (60-80%) and *HMGA1* (5-10%). In lipomas, *HMGA2* but not *HMGA1* fusion genes have been identified. Herein, we describe two novel *HMGA2* fusion genes and the novel *HMGA1-LPP* fusion gene.

Three ordinary lipomas were characterized at the cytogenetic level and showed the following karyotypes: 46,XX,inv(12)(q13q15), 46,XY,add(12)(q24.1), and 46,XX, t(3;6)(q27;p21.1). Frozen tissue material was available from the first two cases but only paraffin tissue was available from the third for further analysis. Fluorescence in situ hybridization(FISH) using custom-designed probes for *HMGA1*, *HMGA2*, and *LPP* were performed to verify the involvement of these loci. RACE RT-PCR, semi-quantitative RT-PCR, and direct sequence analysis were used to confirm the structure and transcriptional upregulation of the fusion transcripts.

Molecular cytogenetic and genetic analyses showed that the lipoma with inv(12) contained a fusion of the *HMGA2* 3-UTR region to a genomic sequence 143kb upstream to the *DCN* locus on 12q21. The lipoma with add(12) had a fusion of the *HMGA2* 3-UTR region to a genomic area 230kb upstream to the *DYRK2* locus on 12q15. RT-PCR and sequence analysis confirmed these chimeric sequences. The lipoma with t(3;6) showed juxta-position of the *HMGA1* to the *LPP* locus, as evident by FISH analysis on paraffin embedded tissue. Semi-quantitative RT-PCR analysis indicated transcriptional upregulation of these genes.

We have identified three novel lipoma fusion genes in ordinary lipoma, including two *HMGA2* fusions and the novel *HMGA1-LPP*. Our findings are consistent with the current hypothesis that suggests deletion or substitution of suppressor sequences in the 3-UTR of *HMGA2* results in its transcriptional upregulation.

Common and cancer type-specific loss-of-heterozygosity (LOH) in the tumor stroma and epithelium of 3 carcinoma types associated with clinical outcome. *M. Orloff, L. Zhang, Y. Xu, C. Eng* Genomic Medicine Institute, Cleveland Clinic, Cleveland, OH.

The role of the tumor microenvironment (stroma) in carcinogenesis and progression has grown in the last decade. We sought to analyze the pattern of in-common and cancer type-specific somatic genomic alterations in epithelium and stroma of breast (BC), prostate (CaP) and head and neck squamous cell (HNSCC) carcinomas. Genomic DNA from tumor epithelium (Ep) and stroma (St) from 413 patients with BC (175), CaP (116) and HNSCC (122) were subjected to 400-microsatellite marker genome-wide LOH analysis. After adjustment for multiple testing (FPRP < 0.5), compartment-specific LOH at a marker was scored as significantly higher (hotpsot) or lower (coldspot) than the average across its relevant chromosome. Notably, there were 16 markers found to be hot/cold-spots of LOH in Ep and/or St which were in-common among all 3 cancer types. These comprised 11 that were St-specific, 2 Ep-specific and 2, D17S2180 and D12S297, which showed significant LOH in both compartments. While we found compartment-specific hot/cold-spot markers of LOH common to all 3 cancer types, there were no hot/cold-spots of LOH specific for any single cancer type only. Overall increased frequencies of LOH across markers in Ep, but not St, correlated with increasing tumor grade (G) for all 3 cancer types. For BC and HNSCC, but not CaP, overall increased St genomic alterations correlated with regional nodal metastases (pN). There were only 2 markers associated with clinicopathologic features that were single cancer type-specific: LOH at D17S2180 in both Ep and St, with G in HNSCC; and LOH at D22S1045 in both compartments with pN. Interestingly, our study reveals that stroma- and epithelial-specific genetic alterations that are in-common among the 3 solid tumors are overwhelmingly more frequent than single cancer type-specific LOH. This suggests common biomarkers for prognosis among several solid tumors. Importantly, our observations help to limit the number of molecular targets for treatment or prevention across several common carcinomas, hence facilitating more precise and compartment-specific therapeutic options.

Cri du Chat mosaicism: a new case with partial deletion and partial deletion/duplication of the short arm of chromosome 5, leading to an unusual phenotype. *A. Nucaro[1], D. Murru[2], MS. Ristaldi[1], L. Boccone[2]* 1) CNR, Istituto di Neurogenetica e Neurofarmacologia, Monserrato, Cagliari, Monserrato, Cagliari, Italy; 2) Dipartimento di Scienze Biomediche e Biotecnologie, Università di Cagliari, Italy.

The Cri du Chat Syndrome(CdCS) is one of the most common deletion syndromes, involving the short arm of chromosome 5, with an incidence of 1 in 50.000 live births. The following are the characteristic features of this syndrome: microcephaly, hypertelorism, round face, micrognatia, epicanthic folds, prominent nasal bridge, hypotonia and severe psychomotor retardation. Patients also show a high pitched cri similar to the mewing of a cat. Deletions and duplications of chromosome 5p have been described in the literature. Mosaicism represents only 3% of this cytogenetic aberration. Up to date, only cases of de novo mosaic 5p anomalies involving two or three rearranged cell lines, with deletions and duplications, have been described. Herein, we report the first case of a patient affected by multiple congenital anomalies and a mosaicism, with two rearranged cell lines: one with a 5p deletion; the other with a 5p deletion/duplication. Our patient did not show the characteristic features described in patients with 5p duplications, but a phenotype compatible with CdCS. Our case represents the first description of a mosaicism with deletion and deletion/duplication of a portion of the short arm of chromosome 5.

Bayesian joint analysis of multiple datasets intended to enhance panic disorder linkage peak on chromosome 7 instead yields evidence of linkage to chromosomes 2q and 17. *M. Logue[1], S. R. Bauver[1], S. P. Hamilton[2], J. A. Knowles[3], A. J. Fyer[4,5], M. M. Weissman[5,6], R. R. Crowe[7]* 1) Genetics Program, Boston Univ Sch of Medicine, Boston, MA; 2) Dept of Psychiatry and Institute for Human Genetics, Univ of California, San Francisco; 3) Keck Sch of Medicine, Univ of Southern California; 4) New York State Psychiatric Institute; 5) Dept of Psychiatry College of Physicians and Surgeons, Columbia Univ; 6) Division of Epidemiology, New York State Psychiatric Institute; 7) Dept of Psychiatry, Univ of Iowa.

Panic disorder is one of the most common anxiety disorders and has substantial comorbidity with other psychiatric disorders and physical ailments. In this paper, we follow-up on our previous findings of a panic disorder region of interest on chromosome 7 obtained using the posterior probability of linkage, or PPL. In Logue et al. 2003, using the 23 pedigrees of the Iowa (IA) linkage data (Crowe et al 2001), we obtained a 2-point PPL of 80% - indicating an 80% chance of a risk locus linked to marker D7S521. Here, we perform a multipoint PPL analysis of the IA data, which increased the peak PPL on chromosome 7 to 88%. These results were combined with a PPL-based analysis of the 120 pedigrees of the Columbia University (CU) genome screen (Fyer et al 2006). Even though earlier analysis with of a subset of the CU data (23 pedigrees, Knowles et al 1998) had provided evidence for linkage to chromosome 7, PPL analysis of the full 120 pedigree CU dataset did not provide further evidence of linkage to this region, and the PPL resulting from joint analysis was 84%. However, joint analysis yielded substantial evidence of linkage to chromosome 2p (PPL = 94% at 74 cM), and chromosome 17 (PPL = 42% at 124 cM), based on strong peaks obtained in the CU pedigrees. There were few regions where joint analysis produced a larger PPL than the individual datasets, most notably, at 39 cM on chromosome 16 (PPL = 25%). This region had been noted previously in the IA data, and has been identified as possibly containing a gene for mitral valve prolapse. The PPL is consistently low elsewhere, with PPLs < 5% for 97% of the genome.

Comparative phenotypic and biochemical analyses of the *Crtap-/-* mice and patients with recessive osteogenesis imperfecta. *R. Morello[1], D. Baldridge[1], J. Lennington[1], T. Bertin[1], E. Munivez[1], M. Jiang[1,5], Y. Chen[1,5], D. Keene[2], D. Rimoin[3], D. Krakow[3], D. Cohn[3], P. Byers[4], B. Lee[1,5]* 1) Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX; 2) Shriners Hospital for Children, Portland, OR; 3) Cedars-Sinai Medical Center and David Geffen School of Medicine, UCLA; 4) University of Washington, Seattle, WA; 5) Howard Hughes Medical Institute, Houston, TX.

We and others have demonstrated that null mutations in either *CRTAP* (Cartilage-associated protein) or *LEPRE1* (coding for Prolyl 3-hydroxylase 1 (P3h1), leprecan) cause lack of type I collagen prolyl 3-hydroxylation and recessive forms of osteogenesis imperfecta (OI). Crtap, P3h1, and cyclophilin B (Prolyl cis-trans isomerase) form a molecular complex in the endoplasmic reticulum (rER) that is responsible for proper collagen post-translational modification. We have now analyzed the stability of this complex in primary human skin fibroblasts derived from patients with null mutations in *CRTAP* or *LEPRE1*. We demonstrate both by immuno-fluorescence and western blot that *LEPRE1-/-* fibroblasts completely lose expression of the CRTAP protein and similarly, *CRTAP-/-* fibroblasts do not express P3H1. These data suggest that both proteins are required in order to form a stable complex in the rER and that lack of either CRTAP or P3H1 cause degradation of the interacting partner. Parallel to these observations, a comprehensive analysis of the phenotype of the *Crtap-/-* mice, a mouse model of recessive OI, shows multiple tissue abnormalities, including in the lungs, kidneys, skin and cartilage. Interestingly, in both the lung and kidney we observe an early onset (P10) increase in cellular proliferation by BrdU staining, suggesting an altered tissue growth rate perhaps due to abnormal matrix to cell signaling. These data suggest CRTAP may exert more widespread effects on collagen homeostasis including types V in skin and type IV in kidney. These studies point to the requirement for prolyl 3-hydroxylation in the widespread regulation of collagen structural and signaling function and suggest a broader clinical assessment of patients with these conditions.

A systematic analysis of the proteins of the human neutrophil phagocytic vacuole. *A. P. Walker[1], M. Radulovic[1,2], A. Sanchez Sierra[1], A. W. Segal[1]* 1) Dept. Medicine, UCL, London, United Kingdom; 2) Dept. Medicine, Imperial College, London, United Kingdom.

Neutrophils comprise the front-line defence of the innate immune system, due to their ability to engulf and degrade invading micro-organisms by phagocytosis. The importance of neutrophil phagocytosis for human health is demonstrated by chronic granulomatous disease, where mutation of subunits comprising the NADPH oxidase complex impairs the respiratory burst that kills microbes, leading to recurrent infections. This study was undertaken to systematically characterise the proteins of the human neutrophil phagocytic vacuole in order to better understand this vital process. Human neutrophils were incubated with latex beads and phagosomes were purified by sedimentation on sucrose density gradients. Proteins from phagosomes or phagosomal membranes were resolved on 1D gels for tryptic digestion and mass spectrometry. 359 proteins were identified by at least two peptides. Notably, 19 RABs were identified including RAB18, RAB35 and RAB8A, which have not previously been recognised in human or mouse neutrophil phagosomes. RABs are small GTPases which act as molecular switches by cycling between GTP- and GDP-bound states. They are important regulators of the different steps of vesicle trafficking. Comparative analysis showed RABs to be the most highly conserved type of protein identified, with nine members conserved to Drosophila melanogaster. ELMO1 (engulfment and motility 1) was identified for the first time in human neutrophil phagosomes; ELMO1 activates rac leading to rearrangement of the actin cytoskeleton for engulfment. The data were scanned for representation of canonical pathway members using GENMAPP software, highlighting the regulation of the actin cytosketon that is essential for the formation and trafficking of the phagocytic vacuole. Ingenuity Pathways Analysis was used to map molecular interactions within the datasets, showing extended networks of cytoskeletal proteins important for vesicle trafficking in phagocytosis.

A general framework for formal tests of interaction after exhaustive search methods with applications to MDR-PDT. *T. Edwards[1], E. Torstenson[2], S. Dudek[2], E. Martin[1], M. Ritchie[2]* 1) Center for Genetic Epidemiology and Statistical Genetics, Dept. of Human Genomics, University of Miami, Miami, FL; 2) Center for Human Genetics Research, Vanderbilt University, Nashville, TN.

As genetic epidemiology looks beyond mapping single disease susceptibility loci, interest in detecting epistatic interactions between genes has grown. The dimensionality and comparisons required to search the epistatic space, the danger of over-fitting, and the inference for a significant result pose challenges for testing epistatic disease models. The Multifactor Dimensionality Reduction Pedigree Disequilibrium Test (MDR-PDT) was developed to test multilocus models in pedigree data. In the present study we rigorously tested MDR-PDT with new cross-validation (CV) and omnibus model selection algorithms by simulating a range of heritabilities, odds ratios, minor allele frequencies, and numbers of interacting loci. Additionally, given that the permutation-based hypothesis test of the MDR-PDT does not evaluate effect modification across genotypes and that this property might inflate the Type I error rate for the null hypothesis of no interaction, we implemented a regression-based permutation test. We found that MDR-PDT performs similarly with 5 and 10 fold CV. We additionally implemented a the matched odds ratio fitness metric and demonstrate that it improves power. We also demonstrate that fitting a regression model on the same data as analyzed by MDR-PDT is a biased procedure and is not a valid test of interaction. The regression-based permutation test implemented here conducts a valid test of interaction after a search for multilocus models, and can be used with any method that conducts a search to find a multilocus model representing an interaction.

Isolation and characterization of Arms2 interacting proteins. *K. Huang, G. Sarawgi, L. Tian, D. Stambolian*
Department of Ophthalmology, FM Kirby Center for Molecular Ophthalmology, University of Pennsylvania, Philadelphia, PA.

Age related Macular Degeneration (AMD) is a common, multi-factorial disease of the macula. Genetic variants at chromosome 1q31-32 and 10q26 are strongly associated with this disorder and may account for as much as 50% of the disease risk. In the 10q26 locus, a strong association has been identified between AMD and SNP rs10490924, which is located within the human ARMS2 gene. ARMS2 encodes a small 12 kD protein that is highly conserved between humans and old world monkeys, both of which have maculas. However, ARMS2 homologs are not present in the genomic sequences of nonprimates that lack maculas, indicating that ARMS2 may play an important role in macular formation. Although ARMS2 is expressed in the retina, retinal pigment epithelium (RPE) and placenta, its function is unknown. To explore the function of ARMS2 in the human retina and RPE, we performed yeast two hybrid screens using full length ARMS2 cDNA as bait and either human retina or RPE/choroid cDNA as prey. We identified three retinal genes that specifically interact with ARMS2: HCHC1 (host cell factor C1, a transcription factor), AFF1 (AF2/FMR2 family member 1) and PTPRD (protein tyrosine phosphatase receptor type D). Characterization of the relationships between ARMS2 and its interacting proteins should provide important information about the function of ARMS2 in macular development and disease.

Neocentromere formation in a stable rearranged chromosome 7 with Class II pericentric interstitial deletion and the formation of ring chromosome 7. *S. A. Ebrahim[1,2], H. Jiang[3], R. Te[2], A. N. Mohamed[1,2]* 1) Detroit Medical Center Univ. Lab. Cytogenetics; 2) Childern Hospital of Michigan; 3) Dept Pathology, Wayne State Univ, Detroit, MI.

Neocentromeres (NEO) are newly derived functional centromeres formed in marker or rearranged chromosome outside the normal centromere location. Since the discovery of the 1st human NEO in 1993, over 90 patients with NEO formation have been reported for all chromosomes with the exception of chromosome 7. We describe a 7 years old boy with developmental delay who has NEO in a previously undescribed chromosome. Routine blood chromosome analysis and FISH revealed a 47,XY,r(7)(p12q21.2),+neo(7)(pterp14neop14p12::q21.2qter)[20].ish r(7)(D7Z1+,WCP7+,ELN+,pter-,qter-),+neo(7)(D7Z1-,WCP7+,ELN- ,pter+,qter+)karyotype. Each metaphase examined had one normal chromosome 7, one chromosome 7 with interstitial deletion of the short and long arms segments[del(7)], and a small ring chromosome derived from the deleted chromosome 7 segments. The ring chromosome stained positive on C-staining, while the del(7) stained negative, indicating the absence of the centromeric region from the del(7). FISH with chromosome 7 centromere specific probe showed that the ring chromosome contained the chromosome 7 centromere and that no alpha-satellite DNA material was present on the del(7), consistent with neocentromere formation. FISH with subtelomeric probes showed the presence of normal short and long arm subtelomere signals on the normal and the del (7), but not the ring 7. FISH with ELN probe for the 7q11.23 region showed positive hybridization, to the normal and ring 7, but no hybridization is observed on the del(7). FISH with whole chromosome 7 paint confirmed the origin of the ring 7 and the del(7) and ruled out an exchange with other chromosome. Ring chromosomes derived from chromosome 7 are rare and a deletion of chromosome 7 with NEO formation has not been reported. This is the first published case of a class II analphoid chromosome derived from chromosome 7, suggesting that this region may possibly be a new site for neocentromere formation.

Autism and germline mosaicism for a *NLGN4* alteration: Genetic counseling implications. *S. Newton[1], G. Zhao[1], H. Tager-Flusberg[2], J. M. Milunsky[1,3]* 1) Center for Human Genetics, BUSM, Boston, MA; 2) Human Development Program, BUSM, Boston, MA; 3) Department of Pediatrics/Genetics and Genomics, BUSM, Boston, MA.

NLGN4 (Neuroligin4) is an X-linked gene located at Xp22.33. The neuroligin (NLGN) family of proteins play a role in synaptogenesis. NLGN mutations have been hypothesized to lead to deficits in cognitive and developmental processes. Mutations in *NLGN4* have been reported in individuals with autism spectrum disorder (ASD), mental retardation, pervasive developmental disorder(PDD), and a variety of neuropsychiatric conditions. We present the first known case of germline mosaicism for a *NLGN4* alteration. The two probands are brothers, ages 5 and 3 yrs, who have been extensively evaluated and found to have classic ASD. They have atypical social interaction, minimal eye contact, difficulty in communication, and repetitive interests and stereotypies. Antenatal courses were unremarkable. Their mother reports no exposures to chemicals, radiation, or severe illness during the pregnancies. Both brothers have developmental delay (especially language), but do not have a history of regression, seizures, neurological trauma, hearing loss, or major medical concerns. Both brothers were found to be hemizygous for the R87W alteration in exon 1 of the *NLGN4* gene. This alteration is not present in the SNP database, was not found in more than 300 normal individuals tested, and is predicted to be possibly damaging by PolyPhen and not tolerated by SIFT. All additional genetic testing on both brothers was negative, including high resolution chromosomes, 500K SNP microarray, Fragile X syndrome, and *NLGN3* sequence analysis. Their mother was found to be negative for the R87W alteration in blood and buccal swabs, and their father was negative for the alteration in blood, ruling out an alteration on the *NLGN4* Y-chromosome homologue. Maternity and paternity was confirmed molecularly. Their mother was found to have mildly skewed X-inactivation (75%:25%) of unknown significance. This report of gonadal mosaicism for a *NLGN4* alteration in 2 brothers with autism has significant implications for genetic counseling and provides further evidence for the causative role of *NLGN4* alterations in the development of ASD.

A Confidendence-Limit Based Approach to the Assessment of Hardy-Weinberg Equilibrium. *S. Wellek[1], K. A. B. Goddard[2,3], A. Ziegler[4]* 1) Dept. of Biostatistics, CIMH Mannheim/University of Heidelberg, Mannheim, Germany; 2) Department of Epidemiology & Biostatistics, Case Western Reserve University, Cleveland, OH, 44106, USA; 3) Center for Health Research, Kaiser Permanente Northwest, Portland, OR, 97227, USA; 4) Institute of Medical Biometry and Statistics, University of Lübeck, Germany.

The classical chi-squared procedure for the assessment of genetic equilibrium is flawed by the fact that it is tailored for detecting violations of HWE although the majority of applications in genetic epidemiology require to establish approximate compatibility of the model assumption with the distribution underlying the data. In Wellek (2004) [Biometrics 60, 694-703], the methodology of statistical equivalence testing was exploited for the construction of tests for problems in which the assumption of approximate compatibility of a given genotype distribution with HWE plays the role of the alternative hypothesis one aims to establish.In the present contribution, we derive a procedure which serves the same purpose but uses confidence limits rather than critical bounds of a significance test of the null hypothesis of absence of marked deviations from HWE. Interval estimation relates to the same parametric function which was previously chosen as the target parameter for constructing an exact conditional UMPU test of equivalence with a HWE-conforming genotype distribution. This population parameter is shown to admit a direct genetical interpretation as a measure of relative excess heterozygosity. For the construction of confidence limits, both asymptotic and exact methods are used.

***APOA2* genetic variation and its relationship with plasma HDL-cholesterol levels.** *M. I. Kamboh[1], S. M. Hollister[1], A. S. Dressen[1], C. H. Bunker[2], R. F. Hamman[3], C. M. Kammerer[1], F. Y. Demirci[1]* 1) Human Genetics, GSPH, Univ Pittsburgh, Pittsburgh, PA; 2) Epidemiology, GSPH, Univ Pittsburgh, Pittsburgh, PA; 3) Preventive Medicine and Biometrics, Univ Colorado Denver, Aurora, CO.

Coronary heart disease is a major public health concern affecting millions of people worldwide. Low levels of high density lipoprotein cholesterol (HDL-C) have been shown to increase the risk for cardiovascular disease. The major genetic loci associated with HDL-C were recently identified through genome-wide association studies, which investigated influences of common variants on common traits. Relatively few studies have investigated the impact of rare variants on common diseases. The aim of our study was to evaluate the role of *APOA2* genetic variation (a biological candidate gene involved in HDL metabolism) in relation to HDL-C levels in epidemiological samples of African Blacks and U.S. non-Hispanic Whites (NHWs). We resequenced the entire *APOA2* gene in selected individuals with HDL-C levels in the upper 5^{th} percentile (47 NHWs and 48 Blacks) and the lower 5^{th} percentile (48 NHWs and 47 Blacks), allowing us to identify both rare and common variants. We detected a total of 26 variants (25 single nucleotide substitutions and 1 microsatellite); 12 of which were previously unreported. Of the 12 new variants, 6 were present in NHWs and 6 in Blacks. We observed an increased number of minor alleles of *APOA2* variants (either increased heterozygosity for rare variants or increased homozygosity for common variants) in subjects with low HDL-C levels that was more pronounced in NHWs. Of the 9 variants that were screened in the larger NHW (n=623; 8 variants) and Black (n=788; 5 variants) samples with TaqMan SNP genotyping assays to date, significant association was found for variants 2233C>T/rs6413453 (p=0.028) and 3251A>G (p=0.023) in NHW females. Complete genotyping of the remaining variants will allow us to determine the extent to which the *APOA2* variants influence the HDL-C levels.

Population-based analysis of copy number variation detects common variants of all sizes. *S. Yoon[1], D. Malhotra[1], M. Kusenda[2], S. Kudaravalli[3], V. Makarov[1], J. Kendall[1], A. Leotta[1], A. Bhandari[1], A. Dewan[7], X. Zhao[1], T. Walsh[4], J. Lloyd[1], B. Yamrom[1], J. Ho[7], A. Singleton[8], J. McClellan[5], M.-C. King[4,6], M. Wigler[1], K. Ye[9], J. Sebat[1]* 1) Cold Spring Harbor Laboratory, Cold Spring Harbor, NY; 2) Graduate Program in Genetics, State University of New York at Stony Brook, NY; 3) Department of Genetics, University of Chicago, Chicago, IL; 4) Department of Medicine, University of Washington, Seattle, WA; 5) Department of Psychiatry, University of Washington, Seattle, WA; 6) Department of Genome Sciences, University of Washington, Seattle, WA; 7) Yale School of Public Health, Yale University, New Haven, CT; 8) Neurogenetics Laboratory, National Institute on Aging, NIH, Bethesda, MD; 9) Department of Epidemiology and Population Health, Albert Einstein College of Medicine, Bronx, NY.

Copy number variants (CNVs) that are individually common in the population are likely to play a role in human traits and disease risk. However, the majority of common variants have not yet been discovered, and little is known about their distribution in different populations. Here we used a population-based CNV discovery method to detect common variation from microarray data. Intensity data from low-resolution and high-resolution microarray CGH platforms were examined from 2 populations, consisting of 2355 and 87 individuals respectively. A pair of oligonucleotide probes was sufficient to detect CNVs with minor allele frequencies of approximately 0.1 and greater. A total of 519 common CNVs were detected with a median size of 2369 bp. 190 CNVs identified were novel polymorphic sites not previously reported in humans and most variants differed strikingly in size and frequency than CNVs previously reported at the same sites. Population data were used to further elucidate patterns of structural variation at individual loci; for instance, in defining the fine-scale structures of individual CNV alleles in a complex region of the genome, and in determining allele frequencies and patterns of linkage disequilibrium (LD) for a subset of CNVs. This method represents an alternative strategy for CNV analysis of population data with greater power to ascertain common variation.

***APOM* sequence analysis in relation to plasma HDL-C levels.** *F. Waqar[1], F. Y. Demirci[1], A. S. Dressen[1], C. H. Bunker[2], R. F. Hamman[3], C. M. Kammerer[1], M. I. Kamboh[1]* 1) Human Genetics, GSPH, Univ Pittsburgh, Pittsburgh, PA; 2) Epidemiology, GSPH, Univ Pittsburgh, Pittsburgh, PA; 3) Preventive Medicine and Biometrics, Univ Colorado Denver, Aurora, CO.

Atherosclerosis is the major cause of morbidity and mortality worldwide. High plasma concentrations of high density lipoprotein cholesterol (HDL-C) are known to protect against atherosclerosis. *APOM* is an apolipoprotein found predominantly in HDL and was reported to have anti-atherogenic functions. The purpose of this study was to examine the impact of *APOM* genetic variation on HDL-C levels in epidemiological samples of African Blacks and American non-Hispanic Whites (NHWs). The entire *APOM* (~2.3 kb) and flanking regions (~1 kb of 5 and ~0.5 kb of 3) were resequenced in selected individuals with HDL-C levels in the upper 5th percentile (47 NHWs and 48 Blacks) and the lower 5th percentile (48 NHWs and 47 Blacks) to identify both common and rare variants. A total of 25 single nucleotide substitutions were identified of which 9 were observed in both ethnic groups and 16 were previously unreported to the best of our knowledge. Of 15 variants identified in NHWs, 4 had minor allele frequency (MAF) of 5% and 11 MAF of <5%. In NHWs, 6 rare variants (MAF <5%) were unique to the low HDL group vs. 2 rare variants unique to the high HDL group. Of 19 variants identified in Blacks, 7 had MAF of 5% and 12 MAF of <5%. In Blacks, equal number of unique rare variants were observed in both the low and high HDL groups (4 in each group). None of the high or low HDL group-unique rare variants identified in Blacks were observed in NHWs. One of the 2 high HDL group-unique rare variants identified in NHWs was more commonly observed in Blacks and had higher MAF in high HDL group as compared to low HDL group. Three of the 6 low HDL group-unique rare variants identified in NHWs were also more commonly observed in Blacks and 2 had higher MAF in low HDL group as compared to high HDL group. Genotyping of these identified variants in the entire set of NHW (*n*=623) and Black (*n*=788) samples, that will help to determine the influence of *APOM* genetic variation on plasma HDL-C levels, is underway.

Genetic analysis of candidate genes for HDL-C in the Iranian INTERHEART study sample reveals associations consistent with European populations. *Z. Dastani[1,2], R. Do[1], M. R. M. Hasani[3], A. Montpetit[4], T. J. Hudson[4], S. Yusuf[5,6], J. Genest[1,2], J. C. Engert[1,2], S. S. Anand[5,6] on behalf of the INTERHEART Investigators* 1) Department of Human Genetics, McGill University, Montréal, QC, Canada; 2) Cardiovascular Genetics Laboratory, McGill University Health Centre, Montréal, QC, Canada; 3) Tehran University, Tehran, Iran; 4) McGill University and Genome Quebec Innovation Centre, McGill University, Montreal, QC, Canada; 5) Population Health Research Institute, McMaster University, Hamilton, ON, Canada; 6) Department of Medicine, McMaster University, Hamilton, ON, Canada.

Coronary artery disease (CAD) is the leading cause of death worldwide and a complex genetic disease. Both environmental and genetic factors lead to atherosclerosis and the subsequent manifestation of clinical disease. HDL-C has been demonstrated to be a strong risk factor for CAD. We examined the possible association between 103 candidate genes and HDL-C in an Iranian study sample. The samples are part of the INTERHEART genetics study that includes individuals enrolled from 154 centers in 52 countries for a global case/control study of acute MI. Previous work by our group established that the genetic background of Iranians was sufficiently different from either Europeans or Arabs that they should be analyzed separately. Several significant associations were found in Iranians. Consistent with previous reports, SNPs in the genes CETP, LPL, ABCA1, SCARB1, LIPC and APOA5 were associated with HDL cholesterol levels (all p values less than 0.007). One significant SNP (rs3764261) in CETP and another (rs18800588) in LIPC had been recently identified in genome wide association studies of HDL-C. In addition, several SNPs in CETP, and LPL were also associated with plasma levels of ApoA1 (p values less than 0.003). This study reveals consistent genetic association results between the INTERHEART Iranian sample and European populations for genes of major effect on HDL-C metabolism.

Complexities in Linkage Analysis for Modifier Genes. *W. Li[1], L. Sun[4,5,1], M. Corey[2,4], R. Dorfman[1], J. Zielenski[1], P. Durie[3], LJ. Strug[2,4]* 1) Genetics and Genomic Bio; 2) Child Health Evaluative Sci; 3) Phys and Exper Medicine; 4) Pub Health Sci; 5) and Statistics, U of Toronto, Toronto, Canada.

Introduction Classic Mendelian diseases e.g. Cystic Fibrosis (CF), are now the subject of modifier studies to understand variation such as disease severity. Blackman et al. (2006) studied Meconium Ileus (MI) in CF families, using non-parametric linkage (NPL) analysis, and found that CFTR, the CF gene, was a determinant of MI. Motivated by their findings, we used NPL and parametric linkage analysis (MMLS, Hodge et al, 1997) in a genome scan of our CF families. MMLS uses trait values robust to misspecification (excluding disease model (MOI)) and uses concordant (C) and discordant (D) MI families. NPL does not require MOI or trait values to be specified, but generally uses affecteds only. We show how NPL can provide confusing results in modifier gene studies. **Methods** We used GENEHUNTER for MMLS and NPL multipoint analyses of MI. 74 families with two CF children were analyzed; 17 of the CF-sibs were C for MI, while 57 were D. **Results** MMLS identified a novel MI locus, D12S1656 (HLOD=2.94), but at D7S522 in CFTR HLOD=0.88. Only 17 families were positive at D7S522, all of whose CF-sibs were C for MI. In contrast, NPL resulted in linkage at both D7S522 (Z=5.26) and D12S1656 (Z=2.11). Using a modified NPL approach that assesses *undersharing* among D sibships as evidence for MI-linkage, we observed *oversharing* (z=9.21) at D7S522 and *undersharing* (z=-2.19) at D12S1656. **Discussion** In modifier studies, siblings C for the modifier trait are C for the disease. Thus, NPL cannot attribute the linkage signal to one phenotype, unless NPL is modified to analyze the Ds for *undersharing* (e.g. D12S1656). Yet this modification cannot detect pleiotropy in modifier studies, because the same locus cannot show both *oversharing* and *undersharing* among the same sibships that are C for the disease but D for the modifier trait (e.g. D7S522). In contrast, parametric analysis can differentiate MI- from CF-linkage and we concluded that D7S522 is linked to CF not MI, and D12S1656 is a novel MI locus. **Conclusion** Care must be taken when interpreting results from standard NPL analysis in modifier gene studies.

Identification and Characterization of genes targeted by ETV6, a transcriptional repressor involved in childhood leukemia. *C. Malouf[1,2], S. Langlois[1], J. Larose[1], D. Sinnett[1,3]* 1) Research Center CHU Sainte-Justine, Montreal, Canada H3T 1C5; 2) Department of Biochemistry, University of Montreal, Montreal, Canada H3T 1J4; 3) Department of Pediatrics, University of Montreal, Montreal, Canada H3T 1C5.

Acute lymphoblastic leukemia (ALL) is a sporadic cancer accounting for about 25% of all pediatric cancer cases. Hemizygous deletions at chromosome 12p12-13 are observed in 26-47% of childhood pre-B ALL cases suggesting the presence of a tumour suppressor gene (TSG) at this locus. Accumulating evidence points to ETV6 as being the most probable TSG targeted by the deletions. ETV6 is a ubiquitously expressed ETS transcription factor with very few known targets. To understand ETV6s function, we previously conducted a microarray analysis that identified 87 genes co-modulated according to ETV6 expression. Among them, the expression of IL18, LUM, SPHK1, TP53 and PTGER4 was significantly correlated with that of ETV6 in leukemia patients. We propose that these genes are direct targets of ETV6. To address this hypothesis, we investigated the promoter activity of all 5 genes in function of ETV6 expression. First, we subcloned the proximal promoters into a gene reporter system (F. luciferase). The promoter constructs were used to perform transient co-transfections in Jurkat and HeLa cell lines with an ETV6 cDNA construct. This suggested that the promoter regions of all 5 genes are direct transcriptional targets of ETV6. These observations are supported by chromatin immunoprecipitation studies indicating an enrichment of ETV6 in the promoters of the 5 genes. Using mutant ETV6 constructs, we discovered that both functional domains, PD and ETS (protein and DNA interactions), are implicated in the transcriptional repression of the genes. This study shows that transcription of IL18, LUM, SPHK1, TP53 and PTGER4 is directly regulated by ETV6 through various molecular mechanisms. We are currently modulating the expression of these genes in leukemia cells to better understand the ETV6 regulation network. This work is another step towards the understanding of the functions of ETV6 and the impact of its inactivation in childhood leukemia.

MFG Maximized: Testing for Disease-Related Maternal-Fetal Genotype Incompatibilities Using the Software Package Mendel. *E. J. Childs[1], T. Ylisaukko-oja[2,3], J. A. Turunen[2], K. Rehnström[2], L. Peltonen[2,3], K. Lange[1], C. G. S. Palmer[1], J. S. Sinsheimer[1]* 1) University of California Los Angeles, Los Angeles, CA; 2) Biomedicum, Helsinki, Finland; 3) University of Helsinki, Finland.

Maternal-fetal genotype (MFG) incompatibility is a gene-gene interaction that adversely affects the developing fetus by inducing a maternal immunological attack, and thereby increase susceptibility to disease. Statistical methods for examining MFG incompatibility as a risk factor for disease using a nuclear family based candidate gene approach have been developed (Sinsheimer et al. 2003, Kraft et al., 2004; Hsieh et al, 2006). Because some of the families collected as part of a study can be large and complex, containing multiple generations and marriage loops, we extend the MFG test to allow for arbitrary family structures. We modify the MFG test by replacing the nuclear-family based mating type approach with Otts representation of a pedigree likelihood, and change the usual Mendelian transmission probability to include a maternal-fetal incompatibility parameter. This computationally tractable approach is implemented using Mendel 8.0 software (Lange et al. 2001), which has an option that allows the user to fit his or her own likelihood. This extension requires a slightly more stringent assumption of random mating that was not necessary in the earlier MFG tests. However the modified test allows for easy inclusion of offspring specific covariates such as offspring genetic risks, sex, age and environmental exposure effects through the penetrance function, to model multiallelic loci as well as biallelic loci, and to use quantitative phenotypes. As a side benefit, this implementation is less data specific and more user friendly than our earlier versions. To illustrate the implementation, we test for the effects of RHD incompatibility on autism spectrum disorders in a data set that includes extended families.

Common Genetic Variants on Chromosome 9p21 Predict Myocardial Injury after CABG Surgery. *K.-Y. Liu[1], J. D. Muehlschlegel[1], T. E. Perry[1], A. A. Fox[1], C. D. Collard[2], S. C. Body[1], S. K. Shernan[1]* 1) Dept Anesthesiology, Perioperative and Pain Medicine, Brigham & Women's Hosp, Boston, MA; 2) Baylor College of Medicine, Div. Cardiovascular Anesthesia, Texas Heart Institute, Houston, TX.

Coronary artery disease is the leading cause of death worldwide. Nearly one million people annually suffer perioperative myocardial injury (PMI) during or after surgery. A recent genome wide association study identified an association between Myocardial Infarction in non-surgical populations and common genetic variants on chromosome 9p21, adjacent to genes for the cyclin-dependent kinases *CDKN2A/B*. We hypothesized that these variants are also responsible for PMI after isolated primary Coronary Artery Bypass Graft (CABG) surgery.

In a prospective observational study of 877 Caucasian CABG patients at 2 US centers, we genotyped 61 haplotype-tagging SNPs, covering 436 kbp of the *CDKN2A* and *CDKN2B* genic region. A multivariable logistic model was used to adjust for previously identified clinical covariates of PMI including severity of coronary disease. Multiple testing of SNPs was corrected for with family-wise (FW) errors.

After CABG, 10% of patients developed PMI, defined as a peak postoperative cTnI greater 8.25 mcg/L. Among the 61 SNPs examined, rs10116277 (G) and rs6475606 (C) are in perfect linkage disequilibrium and describe a single haplotype that is significantly associated with PMI, after accounting for clinical covariates (multiplicative model OR=1.7, 95% CI 1.3-2.4, asymptotic $P=5.7x10^{-4}$, FW empirical P=0.039). The two SNPs are 87 and 72 kbp 5 of *CDKN2A* and *CDKN2B*, respectively. The GC haplotype frequencies for the overall cohort, cases and controls are 41%, 53% and 40%, respectively. Levels of postoperative cTnI clearly showed incrementally increase for each additional copy of the GC haplotype.

In conclusion, we have identified common genetic variants in 9p21 associated with PMI. The functional mechanisms remain to be elucidated.

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Functional Characterization of Human BBS3 Mutations. *D. Y. Nishimura[1], C. C. Searby[2], V. C. Sheffield[1, 2]* 1) Dept Pediatrics, Univ Iowa, Iowa City, IA; 2) HHMI, Univ Iowa, Iowa City, IA.

Bardet-Biedl syndrome (BBS) is a genetically heterogenous autosomal recessive disorder with the primary clinical features of obesity, pigmented retinopathy, plydactyly, hypogenitalism, renal anomalies and learning disabilities. Associated features of BBS include diabetes mellitus, hypertension and congenital heart defects. There are currently 14 BBS genes known that map to 12 different chromosomes. Seven of the BBS genes have been demonstrated to form a complex that has been termed the BBSome. It has been postulated that the BBS proteins are involved in trafficking within cells and cilia. The BBS3 protein ARL6 is part of a gene family that plays a role in the trafficking process. We have utilized the ARPE19 cell line to examine the protein function of both wild type and mutant ARL6 proteins. Native expression of ARL6 has been documented at the centrosome, basal body and within primary cilia in ARPE19 cells. Expression constructs were utilized to express wild type and mutant ARL tagged proteins. All of the ARL6 mutants examined exhibit decreased protein stability and have a strikingly different subcellular localization pattern when compared to wild type ARL6. Therefore, we conclude that the known human mutations in BBS3 are likely to impact both the expression level of the protein as well as potentially interfere with normal cellular localization.

Studies of genome-wide association data support a genetic overlap between type 2 diabetes and prostate cancer.

K. S. Elliott[1], E. Zeggini[1], N. J. Timpson[2], N. W. Rayner[1], C. J. Groves[3], M. I. McCarthy[2] 1) WTCHG, Oxford, UK; 2) Univ of Bristol, UK; 3) OCDEM, Oxford, UK.

Recent genome-wide association (GWA) studies have identified and confirmed many type 2 diabetes (T2D) susceptibility genes. Several of these T2D loci (*PPARG, TCF7L2, CDKN2A, HHEX* and *IGF2BP2*) have known roles in cancer pathogenesis. Association of T2D and prostate cancer (PrCa) at alternate alleles of the same SNP in *HNF1B* support a shared genetic aetiology between the two diseases. Furthermore recent papers report association with T2D and PrCa at separate unassociated variants in the *JAZF1* gene. To formally assess this genetic relationship we compared T2D meta-analysed GWA data from the DIAGRAM Consortium with publicly available GWA PrCa data from the CGEMS project. We searched for more additional cases of reciprocal associations between T2D and PrCa and found subtle association ($p=0.003$, n=16,896 genes) with the opposite allele of the much reported PrCa allele of rs1447295 on 8q24 near the *MYC* gene. Secondly we looked for further instances of genes containing association with both T2D and PrCa and found a signficant over-representation of PrCa signals ($p<0.001$) in genes containing confirmed T2D variants compared to all autosomal genes $p=3x10^{-8}$: in all, six of these regions (*CDKAL1, NOTCH2, CAMK1D, JAZF1, HNF1B* and *KCNJ11*) contained such a PrCa signal. In the reciprocal analysis, there were just the two known T2D signals (*HNF1B* and *JAZF1*) (defined by $p<0.001$) in the 10 confirmed PrCa gene regions ($p=0.102$). For SNPs where there were data available for both diseases (n=393,763), when we looked SNP-by-SNP, genome-wide, for evidence of association signals ($p<0.01$) shared between T2D and PrCa, we found 73 instances representing 42 independent signals compared to an expectation of 8.3 under the null ($p<10^{-6}$). Of these 42 signals, 22 showed discordant and 20 showed concordant directions of association. The non-random co-localisation of T2D and PrCa signals at the genome-wide level indicates undefined overlaps in aetiological mechanisms. The over-representation of PrCa signals in T2D gene regions strengthens the evidence of an overlapping genetic basis for T2D and PrCa.

Genome-wide meta-analysis identify the genes in the regulation of HDL and triglyceride in inbred strains of mice. *Z. Su, M. Leduc, K. Darvishi, R. Korstanje, B. Paigen* The Jackson Laboratory, Bar Harbor, ME 04609.

Many quantitative trait loci (QTL) regulating plasma HDL and triglyceride (TG) concentrations have been identified from independent crosses between inbred strains of mice. We performed a meta-analysis using QTL controlling plasma levels of HDL and TG by assembling a collection of 150 QTL for HDL and 57 QTL for TG from 43 genome-wide scans. The non-redundant QTL were analyzed using the truncated product method. We divided the mouse genome into 100 bins of approximately equal size (~25-30 Mb), and QTL were assigned to the corresponding bins according to their peak locations. Our analysis revealed significant evidence (LOD4.3) of linkage for HDL to 35 regions and TG to 25 regions. For HDL, 9 genomic regions had LOD scores above 20 and 8 had LOD scores between 10-20. For TG, two regions on chromosomes 1 and 2 had LOD scores above 10. Some bins for HDL contained candidate genes for which considerable evidence existed from both studies in the mice and humans, leading us to conclude that these were the HDL QTL genes. Some of the bins with the highest LOD scores contained multiple QTL genes. QTL genes include *Apoa2* on Chr 1, *Apoa1* on Chr 9, *Lipg* on Chr 18, *Lcat* and *Galnt2* on Chr 8, and *Scarb1*, *Mvk*, and *Acads* on Chr 5. Evidence for these and other genes will be reviewed. Our results demonstrated the presence of numerous relatively significant regions that control plasma lipid levels in the mouse. The combinations of human-mouse comparative genomics, haplotyping of mouse strains, gene sequencing and expression studies will help to prioritize and identify the candidate genes.

Genetic Control of Gene Expression at the H1/H2 Locus on Chr17q21 in Patients with Coronary Artery Disease.

K. R. Fitch[1], M. Elashoff[1], S. E. Daniels[1], W. E. Kraus[2], E. R. Hauser[2], S. Rosenberg[1], J. A. Wingrove[1] 1) CardioDx, Inc., Palo Alto, CA; 2) Duke University, Durhan, NC.

Recent studies have shown that gene expression levels in circulating blood cells can be correlated with coronary artery disease (CAD) in patients. The extent to which genetics might influence this correlation however is not clear. We previously measured gene expression in 248 samples from the Duke University CATHGEN registry (a biorepository of clinical and angiographic data in the Duke Databank for Cardiovascular Disease) using 41K Human Whole Genome Agilent microarrays. To identify genes whose expression levels might vary due to underlying genetic differences, a statistical approach was used to discover loci with distinct bimodal expression patterns across the patient population. Eight array features were identified that showed discrete bimodal expression patterns, in addition to being correlated with the extent of CAD. Interestingly, all of these features mapped to 17q21. The ethnic distribution suggested that a genetic component might contribute to the bimodal pattern: 89% of African Americans fell within the lower expressed peak compared to 59% for Caucasians. Targeted genotyping of a subset of the CATHGEN patients (n=144) identified a SNP (rs9468) that was highly correlated with gene expression at these 8 loci (p value range 1.5E-09 to 2.81E-57). Further analysis revealed that rs9468 is linked to a previously described 900kb inversion, referred to as the H1/H2 locus, which is flanked by several copy number variable sites (CNVs). To investigate the relationship between the H1/H2 haplotype and gene expression, RT-PCR was used to assess both gene expression and copy number for 8 genes in the interval. The expression patterns of 4 genes in the central single copy portion (no CNVs) were independent of haplotype, whereas the expression of 4 genes that overlapped CNVs were highly associated with H1/H2 allele status. This is one of the first descriptions of gene expression changes associated with the H1/H2 locus at 17q21. Further studies will be needed to elucidate the molecular basis of the genetic influence on gene expression at this locus.

Using transmission rates in family-based genome-wide association studies to estimate platform-specific genotyping errors that cannot be detected by standard quality control filtering. *D. Fardo[1], R. Tanzi[2], L. Bertram[2], S. Weiss[3], D. Pinto[4], S. Scherer[4], C. Lange[1, 3]* 1) Dept Biostatistics, Harvard Sch Public Health, Boston, MA; 2) Dept of Neurology, Massachusetts General Hospital, Harvard Medical School, Charlestown, MA; 3) Channing Laboratory, Harvard Medical School, Boston, MA; 4) Centre for Applied Genomics, University of Toronto, Toronto.

An intuitive way to assess the genotyping error rate of genome-wide SNP chips that will persist in the data, even after strict data quality control (QC) filters have been applied, is to utilize family samples that have been genotyped on the same platform. Since the null hypothesis of no association will be true for the vast majority of SNPs on a chip, the number of SNPs for which the minor allele is over-transmitted should be about the same as the number of SNPs for which the major allele is over-transmitted. If the two transmission numbers are very different, this can be indicative of genotyping errors at a platform-wide level after QC filtering or of differential missing genotypes. In this communication, we provide guidelines on how to assess the magnitude of the underlying platform-wide genotyping error based on the transmission ratio for a given number of genotyped SNPs and given the study sample size. The dependence of the transmission ratio on the genotyping error and the percentage of missing genotypes is examined. We illustrate the importance of the transmission ratio as a QC assessment tool in genome-wide association studies by estimating the systematic genotyping error of some of the most commonly used genotyping platforms (Affymetrix 20K, Affymetrix 100K, Affymetrix 500K, Perlegen 650K and Illumina 550K).

Multivariate genome-wide linkage scan of age at menarche and contemporaneous skeletal age and body mass in healthy girls from the Fels Longitudinal Study. *B. Towne[1], J. Blangero[2], S. A. Czerwinski[1], E. W. Demerath[3], D. L. Duren[1], K. D. Williams[1], T. D. Dyer[2], S. A. Cole[2], R. J. Sherwood[1], W. C. Chumlea[1], R. M. Siervogel[1]* 1) Wright State University Boonshoft School of Medicine, Dayton, OH; 2) Southwest Foundation for Biomedical Research, San Antonio, TX; 3) University of Minnesota, Minneapolis, MN.

A complex series of orchestrated processes comprise normal growth and development, and different measures of growth and development are often correlated. We used variance-components methods implemented in SOLAR (Almasy and Blangero, 1998) to conduct univariate and multivariate genome-wide linkage scans for QTL influencing age at menarche (AAM) and contemporaneous skeletal age (SA) and body mass index (BMI) in 321 healthy girls from 61 families in the Fels Longitudinal Study. AAM data were obtained from questionnaires administered at exams conducted regularly throughout childhood, estimates of SA were made from hand-wrist radiographs using the FELS method (Roche et al., 1988), and BMI was calculated as kg/m^2. SA and BMI at AAM were derived by linear interpolation using data from the two exams most closely bracketing AAM. The heritablities of AAM, SA@AAM, and BMI@AAM were all significant: 0.52 0.16, 0.80 0.14, and 0.66 0.18, respectively. The highest multipoint LOD scores for each trait considered individually were: AAM LOD = 1.47 on chromosome 11 at 72 cM, SA@AAM LOD = 1.25 on chromosome 18 at 103 cM, and BMI@AAM LOD = 2.21 on chromosome 3 at 42 cM. A multivariate linkage analysis of all three traits considered simultaneously, however, revealed a significant LOD score of 3.51 on chromosome 7p at 39 cM, and a suggestive LOD score of 2.39 on chromosome 10q at 101 cM, two chromosomal regions not strongly implicated in univariate linkage analyses. This study illustrates the power of multivariate genome scans of correlated measures to uncover QTL harboring genes that influence sets of related traits, in this instance genes that impact a suite of related traits pertaining to the timing and tempo of growth and maturational events during female pubertal development. Supported by NIH grants R01HD12252, R01HD36342, and R37MH59490.

A Tangled Web: Exclusive Patent Rights and Genetic Testing for Long QT Syndrome. *M. Angrist, S. Chandrasekharan, C. Heaney, R. Cook-Deegan* Institute for Genome Sciences & Policy, Duke University, Durham, NC.

What effects do gene patents have on access to and quality of genetic testing? We consider Long QT syndrome, which causes the hearts electrical system to malfunction and whose symptoms may include fainting, seizures, arrhythmia and cardiac death. Some 70% of LQTS is due to mutations in 5 genes; knowing the mutant gene can have a major impact on therapy. Patent rights to the 5 genes are exclusively licensed to PGx Health (PGx). In 2007 a reference lab CEO and a medical geneticist testified before Congress that LQTS patent exclusivity is anti-competitive and quality has suffered. The geneticist said that PGx's testing has on occasion been incomplete and/or inaccurate. Both contended that the $5400 test is overpriced and is not routinely covered by 3rd-party payers. They charged that there was a 2-year period when commercial genetic testing was not available and patients may have died as a result. The reference lab said it tried to sublicense the LQTS patents and was rebuffed by PGx and prior licensees. PGxs parent firm, Clinical Data (CLDA), maintained that only through exclusive licensing has it been able to invest in and develop accurate and safe genetic testing for LQTS. CLDA said it works closely with academics to ensure that its test is state-of-the-art. It said it requires at least 2 clear analyses for every test and that all positives are confirmed. Its LQTS test is covered by Medicare and many private insurers and state Medicaid programs. CLDA also suggested the complaining lab was being disingenuous since said lab had approached CLDA about buying its lab unit and recently obtained an exclusive license to another LQTS gene. After interviewing the major stakeholders, we conclude that commercial LQTS testing is tightly linked to gene patents and licenses. The sole provider model, as embodied by PGx, results in other labs being excluded. Exclusive licensing of different genes to different labs can lead to cross-licensing or litigation. Disparate payer policies can obstruct access to genetic testing when contracts do not cover needy patients. This can happen even without patenting, but patents can exacerbate the problem by granting exclusive rights.

Clinical and molecular analysis of PTEN promoter variant -1084C/T in 22 families with PTEN Hamartoma Tumor Syndrome (PHTS): mutation-specific genetic counseling. *E. Edelman, C. Eng* Genomic Med Inst, Cleveland Clinic Fndn, Cleveland, OH.

PHTS comprises several different syndromes such as Cowden syndrome (CS) and Bannayan-Riley-Ruvalcaba syndrome (BRRS) characterized by PTEN mutations and a risk of breast and thyroid cancers. In ~10% of CS, a mutation is identified in the PTEN promoter. PTEN promoter variant of unknown significance (VUS) -1084C/T was previously reported in 3/751 (0.4%) white population controls. We report on a series of 22 families with -1084C/T and otherwise normal PTEN sequence. 12/18 informative probands met clinical diagnostic criteria for CS/BRRS. The remaining families had some PHTS features but did not meet full criteria. All 15 females had breast involvement of which 14 were carcinoma. Patients with -1084C/T were more likely to have breast cancer (p=0.0008) and less likely to have macrocephaly (p=0.0001), benign thyroid disease (p=0.0001), and GI (p=0.034) and cutaneous hamartomas (p=0.0001) compared to other CS/BRRS without this VUS. Interestingly, 3 patients were reported to have papillary thyroid carcinoma rather than the typical follicular type in PHTS. Compared to the general population, patients with -1084C/T were more likely to have macrocephaly (p=0.0001), breast cancer (p=0.0001), and lipomas (p=0.0018). PTEN downstream dysfunction was characterized by decreased PTEN protein and/or increased P-Akt and P-MAPK. Family analysis was possible in 2 cases: in 1, the VUS was de novo in the context of clinically unaffected parents, and in another, inherited from an unaffected father. -1084 is highly conserved among species and results in reduced binding of p53 to the PTEN promoter. Therefore, our integrative clinical, genetic and functional molecular analyses suggest that -1084C/T is a pathogenic promoter mutation associated with a particularly high risk of breast cancer compared to all intragenic PTEN mutations. Additionally, if -1084C/T patients develop thyroid cancer, it tends to be papillary instead of the more typical PHTS-associated follicular histology. Further work along these lines will garner accurate risk data to allow for mutation-specific genetic counseling.

Gene Expression Responses to Endoplasmic Reticulum (ER) Stress in Humans. *R. S. Spielman[1], B. A. Dombroski[1], W. M. Ankener[1], K. A. Chapman[2], K. G. Ewens[1]* 1) Dept of Genetics, Univ Pennsylvania Sch Medicine, Philadelphia, PA; 2) Division of Genetics, The Children's Hospital of Philadelphia, Philadelphia, PA.

The cellular response to endoplasmic reticulum (ER) stress is a key process in cell survival. The responses, including changes in gene expression, are crucial to cell function; if ER homeostasis cannot be restored, the cell undergoes apoptotic cell death. Identifying determinants of genetic variation in ER stress response will contribute greatly to understanding the process and its role in health and disease. Here we address two questions: 1. Which genes show the greatest expression responses to ER stress, and 2. Which expression responses show heritable variation? The experiments were carried out with lymphoblastoid cell lines from 14 Caucasian and 12 African American monozygotic (MZ) twin pairs. We induced ER stress with thapsigargin or tunicamycin and analyzed gene expression with Affymetrix U133 Plus 2.0 arrays. Among the 986 transcripts with response greater than 1.5-fold, some were previously known to be involved in ER stress; examples are DDIT3/CHOP (fold-change 3.2), CEBP/B (5.3) and TRIB3 (4.6). In addition, there were strong expression responses by some genes not known to be ER stress responsive, e.g., INHBE (14.4 fold) and VLDLR (10.9). The changes in more than 700 of the 986 transcripts were statistically significant even after Bonferroni correction. Some, but not all, of the new findings can be accommodated within current understanding of ER stress pathways. For assessment of heritable variation, the gene expression changes in six of the Caucasian MZ twin pairs treated with tunicamycin were analyzed by analysis of variance (ANOVA). From the ANOVA we also calculated the intraclass correlation coefficient (ICC). We used the ICC to rank the genes for heritable variation in expression response to ER stress. Among those with high ICC were several known to be involved in ER stress: IRE1/ERN1 (ICC= 0.82), HERPUD1 (0.97), ATF4 (0.89), and ATF3 (0.86). There are also genes with high ICC that have not previously been implicated in ER stress. This analysis provides evidence for genetic variation in gene expression response to ER stress.

Capture-recapture methods for counting copy-number variants in the human genome. *I. Ionita-Laza, C. Lange, N. Laird* Dept Biostatistics, Harvard Univ, Boston, MA.

Copy-number variation represents a substantial source of genetic variation and has recently emerged as a potential contributor to disease risk. The number of common copy-number variants and their frequencies are of great interest for human genetic applications; they are currently unknown, and subject to speculation. In this paper we provide a method to estimate the number of common copy-number variants, assuming high-quality data is available on a small set of at least two individuals. Our method is related to capture-recapture techniques commonly used in ecology to estimate the size of a particular species. An application of the methodology to paired-end sequence data on two individuals illustrates that while the number of rare copy-number variants can be very large, the number of common copy-number variants is small and, surprisingly, can be estimated from data on a few individuals. The method is particularly relevant in the context of the ``1000 Genomes Project'' which will produce deep sequence data for two trios by the end of the year. When data on many more individuals is available, we present methodology that provides estimates of the number of new variants to be discovered in a new dataset of a specified size.

Frequency of Copy Number Variants (CNVs) in Ashkenazi Jewish Parkinsons disease Patients and Controls. *M. Verbitsky[1], S. Kisselev[2], M. Wei[2], H. Mejia-Santana[4], H. Andrews[4,5,6], S. Fahn[7], J. Lee[4], K. Marder[1,4,5,7], L. N. Clark[1,2,3]* 1) Taub Institute, Columbia University, New York, NY; 2) Department of Pathology, Columbia University, New York, NY; 3) Center for Human Genetics, Columbia University, New York, NY; 4) G. H. Sergievsky Center, Columbia University, New York, NY; 5) Department of Psychiatry, Columbia University, New York, NY; 6) Department of Statistics, Columbia University, New York, NY; 7) Department of Neurology, Columbia University, New York, NY.

Studies of CNVs affecting the SNCA (PARK1) and Parkin (PARK2) loci in familial Parkinsons disease (PD) have shown that CNVs at genes associated with PD can cause or modify the disease phenotype. A recent study (Simon-Sanchez et al, 2008), identified CNVs by analyzing a dataset from a genome wide SNP scan in a cohort of unique and unrelated individuals comprised of PD patients and controls from a Caucasian population. CNVs in both PD patients and control subjects were detected in PARK2. However, no structural alterations were observed at known PD loci including PARK1, DJ_1 (PARK7), PINK1 (PARK6) or LRRK2 (PARK8). In the current study we set out to find CNVs that may contribute to PD susceptibility by performing a genome-wide scan of CNVs in early onset PD (EOPD) patients and controls from a genetically isolated population, Ashkenazi Jews (AJ). Such population is more likely to be enriched for founder mutations, increasing the power of a case-control study in revealing susceptibility alleles. We used Illumina Human610-Quadv1.0 DNA BeadChips, which include 550,000 evenly spaced tag SNPs derived from HapMap data in addition to approximately 60,000 additional markers that specifically target regions known or likely to contain CNVs. A total of 200 subjects that includes 100 AJ EOPD patients and 100 AJ controls were genotyped. PD patients and controls were a subset of participants in the NY PD AJ study (NS050487). All patients and controls have been previously evaluated for mutations in identified PD susceptibility genes including Parkin, DJ_1, PINK1, GBA and LRRK2. Biostatistical analysis of the data is currently in progress and its results will be presented in the study.

Cytogenetic characterization of a dermatofibrosarcoma protuberans with a complex karyotype including an unbalanced der(22)t(17;22)(q22;q13). *N. Christacos[1], T. Tchen[1], J. Jahn[1], J. Scheerle[1], J. Meck[1], J. Mize[2], F. Wodajo[3], P. Mowrey[1], A. Meloni-Ehrig[1]* 1) Quest Diagnostics, Nichols Institute, Chantilly, VA; 2) Inova Fairfax Hospital Lab, Falls Church, VA; 3) Inova Fairfax Hospital and Hospital for Children, Fairfax, VA.

We report a unique case with an unbalanced t(17;22)(q22;q13) in a 51 year old female with the clinical indication of sarcoma of the upper left back. The pathology report suggested findings most consistent with a high grade sarcoma although a dermatofibrosarcoma protuberans (DFSP) with sarcomatous transformation had been considered. Conventional G-band chromosome analysis was performed and revealed a complex abnormal karyotype including two copies of a derivative chromosome 22 arising from a translocation between chromosomes 17 and 22 [der(22)t(17;22)(q22;q13)]. Additional FISH studies were performed on metaphase cells using the LSI 22 (BCR) probe and probes to the subtelomeric region of the long arm (q) of chromosome 17 and confirmed the presence of two copies of the derivative chromosome 22. Interestingly, the FISH studies also identified a cryptic rearrangement of chromosome 9 as probes for the subtelomeric regions of the short and long arms of chromosome 9 are included with the subtelomeric probe for 17q. The t(17;22) has been reported in cases of DFSP; however, the majority of DFSP cases reported have shown excess of sequences of chromosomes 17 and 22 usually in the form of supernumerary ring chromosomes or marker chromosomes. Fusion of the COL1A1 gene at 17q21~q22 and the PDGFB gene at 22q13 has been shown to occur in both the ring and translocated chromosomes. Thus, these cytogenetic findings were crucial to providing the specific diagnosis of DFSP with sarcomatous transformation in this patient. This case demonstrates the importance of conventional cytogenetic analysis as well as molecular cytogenetic FISH studies in providing critical information to help arrive at an accurate diagnosis and therefore determining treatment and clinical prognosis.

Accuracy of Genome-Wide Imputation of Untyped Markers and Impacts on Statistical Power for Association Studies. *K. Hao, E. Schadt* Genetics, Rosetta Inpharmatics, Seattle, WA.

Genotyping arrays employed by WGAS only assay a small proportion of SNPs in the human genome. In addition, various SNP arrays assay different sets of SNPs, causing challenges in comparing results and meta-analysis. Genome-wide imputation of untyped markers addresses these issues in a direct fashion. 384 Caucasian American liver donors were genotyped using Ilmn650Y arrays. In parallel, 200 Caucasian American and 200 African American subjects were genotyped using the Affymetrix SNP 6.0 array. We compared two genotype imputation methods: MACH and BEAGLE, and found them to perform similarly with respect to imputation accuracy. The Affx6.0 and Ilmn650Y results were comparable, and they give better imputation results than Affx500K or Ilmn317K sets. In Caucasians, 90% of the 2.5 million HapMap release22 SNPs were imputed at 98.5% accuracy. As expected, imputation of poorly tagged SNPs (untyped SNPs in weak LD with typed markers) was not as successful. It was more challenging to impute genotypes in the African American, which is likely due to (1) shorter LD blocks and (2) populations admixture. To address issue (2), we pooled HapMap CEU and YRI data as an imputation reference set, which greatly improved overall performance. 80% of the HapMap SNPs were imputed at 97.5% accuracy in the African American. We measure ~40,000 liver mRNA expression traits for the liver donors and conducted eQTL mapping using imputed SNPs. The ~40,000 phenotypes scored provide a path to empirically determine how the power to detect associations is affected by using imputed SNPs vs. assayed SNPs only. That is, at a fixed false discovery rate, the number of cis-eQTL discoveries detected by various methods can be interpreted as their relative statistical power in GWAS. We find that imputation improves power by 5% for studies using the Ilmn317K array, but decreases power by 8% for Ilmn650Y studies. This shift in power is likely due to the dense SNP arrays already covering most of the genetic variation in a population, so that adding more markers via imputation results in little gain in genetic coverage, but a significant gain in the multiple testing penalty.

Screening and replication using the same data set: Testing strategies for family-based studies in which all probands are affected. *A. Murphy*[1,2,3], *S. T. Weiss*[1,2,3], *C. Lange*[1,3,4] 1) Department of Medicine, Harvard Medical School, Boston, MA; 2) Channing Laboratory, Brigham and Women's Hospital, Boston, MA; 3) Center for Genomic Medicine, Brigham and Women's Hospital, Boston, MA; 4) Department of Biostatistics, Harvard School of Public Health, Boston, MA.

For genome-wide association studies in family-based designs, we propose a powerful two-stage testing strategy that can be applied in situations in which parent-offspring trio data are available and all offspring are affected with the trait or disease under study . In the first step of the testing strategy, we construct estimators of genetic effect size in the completely ascertained sample of affected offspring and their parents that are statistically independent of the family-based association/transmission disequilibrium tests (FBATs/TDTs) calculated in the second step of the testing strategy. For each marker, the genetic effect is estimated(without requiring an estimate of the SNP allele frequency) and the conditional power of the corresponding FBAT/TDT is computed. Based on the power estimates, a weighted Bonferroni procedure assigns an individually adjusted significance level to each SNP. In the second stage, the SNPs are tested with the FBAT/TDT statistic at the individually adjusted significance levels. Using simulation studies for scenarios with up to 1,000,000 SNPs, varying allele frequencies and genetic effect sizes, the power of the strategy is compared to standard methodology (e.g., FBATs/TDTs with Bonferroni correction). In all considered situations, the proposed testing strategy demonstrates substantial power increases over the standard approach, even when the true genetic model is unknown and must be selected based on the conditional power estimates. The practical relevance of our methodology is illustrated by an application to a genome-wide association study for childhood asthma, in which we detect two markers meeting genome-wide significance that would not have been detected using standard methodology. Funding: U01 HL065899-06, U01 HL065899 and P01 HL083069.

Effects of stage of reproduction, nutrient status, and genes on serum homocysteine in reproductive age women. *S. K. Shapira[1], A. Yesupriya[2], J. Robitaille[1], R. Fisk Green[1], H. C. Hamner[1], J. E. Kimmons[3], K. S. Crider[1]* 1) NCBDDD, CDC, Atlanta, GA; 2) NOPHG, CDC, Atlanta, GA; 3) NCCDPHP, CDC, Atlanta, GA.

Elevated serum homocysteine (Hcy) has been associated with adverse pregnancy outcomes, such as preterm birth, stillbirth, and low birth weight, as well as with cardiovascular disease and stroke. To evaluate genetic and environmental factors affecting Hcy, our study utilized survey data of 2,012 reproductive age women (17-44 years) from the NHANES III DNA Bank (1991-94). Associations between genetic variants (MTHFR 1298A-C, 677C-T, and 116C-T, MTRR 66A-G, and CBS 844ins68) and Hcy were tested using linear regression models that adjusted for demographic, reproductive, dietary, and environmental factors. To examine possible effect modification, models were stratified by race/ethnicity, reproductive stage in relation to pregnancy (never, currently, 2 years, >2 years), and total folate intake (low vs. adequate, accounting for both folic acid from vitamins and food folate). Significant associations with mean Hcy were detected only for MTHFR 677C-T. Mean Hcy among TT women was higher compared with CC women (9.5 vs.7.1 mol/L, $p<0.001$). When stratified by total folate intake, TT women with low intake had higher mean Hcy, compared with CC women (16.7 vs. 7.6 mol/L, $p<0.001$), while TT and CC women with adequate intake had similar mean Hcy (7.1 vs. 6.8 mol/L, $p=0.44$). Mean Hcy differed by genotype (TT vs. CC) for whites (17.9 vs. 7.7 mol/L, $p=0.003$) and Mexican Americans (8.8 vs. 6.5 mol/L, $p=0.006$) with low total folate intake, although the difference was smaller for the latter. Models could not be stratified for blacks because of the low TT prevalence. A similar genotype effect was seen for women with low total folate intake in all reproductive stages except currently pregnant, where TT women had lower mean Hcy compared with CC women (4.9 vs. 7.0 mol/L, $p=0.003$). These results suggest that the effect of total folate intake on the association between MTHFR 677C-T and Hcy is modulated by stage of reproduction and race/ethnicity. Therefore, adequate total folate intake appears to be important for reproductive age women, particularly those with the MTHFR 677TT genotype.

Single nucleotide polymorphisms (SNPs) in DNA repair pathway genes may play a role in breast cancer. *M. E. Sehl, L. R. Langer, J. C. Papp, L. Kwan, J. L. Seldon, G. Arellano, J. Reiss, E. F. Reed, S. Dandekar, Y. Korin, J. S. Sinsheimer, Z. F. Zhang, P. A. Ganz* University of California, Los Angeles.

DNA damage recognition and repair is well known to play a major role in cancer risk. We investigated 104 SNPs in 17 genes whose protein products are involved in double strand break repair (DSBR). We used a case-control study design involving 399 participants of the UCLA Familial Cancer Registry. The cases were diagnosed with either breast cancer alone or with both breast and ovarian cancer; and the controls had similar familial risk of breast cancer and were well-matched for demographic and environmental risk factors for breast cancer. Log additive and dominant models were used to investigate associations between SNPs and breast cancer, with models adjusted for age, education and Ashkenazi Jewish ancestry. Haplotype analyses were carried out on genes in which more than one SNP was found to be significantly associated with breast cancer. We found that 12 of the polymorphisms from 8 genes (XRCC4, XRCC2, NBS1, RAD21, TP53, BRIP1, and ZNF350) are associated with breast or breast and ovarian cancer. Most notable were three SNPs located in introns of RAD21: rs16888927 (aOR 0.51, p=0.004), rs16888997 (aOR 0.56, p = 0.002) and rs16889040 (aOR 0.013, p = 0.013), using dominant genetic models. When only those participants with known BRCA1 and BRCA2 status (N=306) were included, these three SNPs remained significant in both log additive and dominant models adjusted for BRCA1 and BRCA2 status. Using a false discovery rate of 15%, we find that 3 SNPs from RAD21 and 1 from BRIP remained significant after adjusting for multiple testing. There was an association found between the C-A-A haplotype for RAD21 (aOR 1.6, p = 0.0068), although this did not surpass the level of significance of the effects of single SNPs in this region alone. We conclude that SNPs within or near a number of DSBR DNA repair pathway genes are associated with breast cancer in individuals from a high-risk population, suggesting that other genes in the DSBR pathway in addition to BRCA1 and BRCA2 may affect breast cancer risk.

Complexity at breakpoint junctions of 1p36 interstitial deletions indicates mechanisms of formation. *M. Gajecka*[1], *A. J. Gentles*[2], *K. L. Mackay*[1], *C. Glotzbach*[1], *L. G. Shaffer*[1] 1) School of Molecular Biosciences, Washington State University, Spokane, WA; 2) School of Medicine, Stanford University, Stanford, CA.

Deletions of 1p36 occur in approximately 1 in 5,000 newborns. To date, we have ascertained 145 cases with monosomy 1p36, representing four possible classes of rearrangements: pure terminal deletions, interstitial deletions, unbalanced translocations, and complex rearrangements. For each individual, the type of rearrangement, deletion size, and parental origin of the deletion was determined using array CGH, FISH and genotyping. To further understand the mechanisms for chromosome rearrangements, we investigated the breakpoint junctions at the sequence level in more than 20% of our cases. Here we present the breakpoint junction evaluations in 18 interstitial deletions. Among these cases, in addition to the major deletion, further events were identified, including small deletions, duplications, and triplications, insertions of both unidentified and known sequences, and inversions at the breakpoint junctions. Our results show high complexity at the breakpoint junctions and indicate involvement of multiple mechanisms in the DNA breakage and repair process during rearrangement formation. Although the causes of the chromosomal breaks that initiate the interstitial deletion formation are unknown, the joining mechanisms are consistent with features of the nonhomologous end-joining (NHEJ) pathway.

Association Mapping of Brain Gene Expression Regulators. *C. Liu[1], L. Cheng[1], JA. Badner[1], DW. Craig[3], M. Josephson[3], SL. Christian[2], ES. Gershon[1,2]* 1) Department of Psychiatry, The Univ Chicago, Chicago, IL. 60637; 2) Department of Human Genetics, The University of Chicago, Chicago, IL 60637; 3) The Translational Genomic Research Institute, Phoenix, AZ 85004.

Gene expression variation may be involved in complex disease risk. Linkage and association methods have been used to map expression regulatory elements in the genome. We performed a genome-wide SNP based association mapping of gene expression regulators, using DNA from 135 specimens in the Stanley Medical Research Institute (SMRI) brain collection of Caucasian brain samples. Affymetrix 5.0 was used to obtain genotypes. Gene expression data of prefrontal cortex was produced using Affymetrix Human Genome U133A arrays, and published by Altar et al. Expression of 3431 transcript probes with variable expression was correlated with high quality genotypes of 238389 SNPs with minor allele frequency no less than 0.1. Association tests were performed separately in cis- and trans- candidate regions. We used the SOLAR program to estimate a sporadic model for the expression data of each probe incorporating covariates provided by SMRI, including age, gender, brain pH, and others. The residuals were the phenotypes for association analysis. We then used PLINK to perform linear regression analysis to test for quantitative trait locus association between expression residuals and SNP genotypes. The Wald p-value and permutation were used to evaluate the significance of association. All region-wide/genome-wide significant cis and trans findings were also analyzed for additive and epistatic interactions. 757 cis- associations had $p < 0.05$ with permutation corrected for region-wide tests. 114 of these are significant after Bonferroni correction for the phenotypes that have been tested. Cis- regulations of genes HBS1L, RPS26, ITGB3BP, and C9orf95 replicate previous findings. 158 trans- associations showed association after permutation correction for the SNPs tested. But none survived correction for the number of expression phenotypes. Potential master regulators, epistatic and additive interactions were also observed. The identified expression regulators were further explored in disease association data.

A test for genetic association that incorporates information from other sources. *J. Wang, S. Shete* Epidemiology, UT MD Anderson Cancer Center, Houston, TX.

To assess genetic association between single-nucleotide polymorphisms (SNPs) and disease status, the logistic regression model or generalized linear model is typically employed. However, information from other resources could also be used to construct a more powerful test, such as information from markers from the same genetic region. To develop a more powerful statistical test for genetic association studies, we quantified the significance of the information from these other sources, and integrated it to the standard regression approaches that use cases and controls. Results from simulation studies and real disease studies demonstrate that the new approach is more powerful than the traditional logistic regression model. And the type I error probabilities of our approach were also well controlled. For our approach, we derived exact formulas to compute p-values. Although the exact p-values are simple and straightforward to compute and interpret, the derivations of underlying assumptions might make the exact p-values based on the explicit formulas either too conservative or too liberal. Therefore, we also developed an approach to estimate empirical p-values using a re-sampling procedure.

A variant in the telomerase RNA component is associated with short telomere length. *O. T. Njajou[1], L. Pawlikowska[1], D. S. Evans[1], N. Bendjalali[1], P.-Y. Kwok[1], E. H. Blackburn[1], A. B. Newman[2], G. Tranah[1], M. Nalls[3], S. Kritchevsky[4], T. B. Harris[3], R. M. Cawthon[5], W.-C. Hsueh[1] For the Health ABC Study* 1) University of California, San Francisco, CA; 2) University of Pittsburgh, Pittsburgh, PA; 3) NIA, Bethesda, MD; 4) Wake Forest University, Winston-Salem, NC; 5) University of Utah, Utah, UT.

Telomeres are DNA capping structures protecting the ends of mammalian chromosomes, which shorten as the cell divides. This shortening is compensated for by the enzyme telomerase. We evaluated the association of polymorphisms in the telomerase RNA component (TERC) with telomere length (TL) in 3075 participants in the Health ABC study (age range: 68-80 years, 51% female, 1794 white and 1281 black). Five variants were identified in TERC by sequence analysis covering the entire gene of 451 bp. From these, only one (rs2293607, G/A) had a minor allele frequency (MAF) > 5%, (A allele, MAF = 0.24 in white and 0.07 in black). Telomere length was measured by quantitative PCR (qPCR) method. Genotyping and TL measurements in leukocytes were successful in 2620 individuals (1542 white and 1078 black). Modeling of the association of SNP genotype with TL by analysis of variance and linear regression adjusting for age, sex, recruitment sites and race revealed that a dominant model for the minor allele was a best fit to the data. Around 42% (655) of white and 14% (148) of black participants were carriers of the A allele. The mean TL in kilo base-pairs (kbp) was significantly shorter in carriers of the A allele compared to non-carriers among whites; 4.86 0.04 kbp vs. 4.69 0.05 kbp (P = 0.009); but not among blacks; 4.88 0.04 kbp vs. 4.84 0.1 kbp (P = 0.7). There was a significant association between TL and the AA/AG genotypes in whites (adjusted beta = -0.17 + 0.06 Kbp, P = 0.005), but not in blacks (adjusted beta = -0.04 + 0.10 Kbp, P = 0.7). Our results in blacks may be underpowered due to the low frequency (0.07) of the A allele in this population. We found that a common variant in the TERC gene is associated with telomere length, which suggests that TERC may play a role in telomere homeostasis in normal blood cells.

SNPs in Ly9 gene observed no association with rheumatoid arthritis in Japanese population. *A. Suzuki[1], Y. Kochi[1], E. Kanno[1], K. Kobayashi[1], R. Yamada[1, 2], K. Yamamoto[1, 3]* 1) Ctr for Genomic Med, Kanagawa, RIKEN, Yokohama City, Japan Akari Suzuki; 2) Laboratory of Functional Genomics and Molecular Medicine, Human Genome Center, Institute of Medical Science, The University of Tokyo, Tokyo, Japan; 3) Dept of Allergy and Rheumatology, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan.

Multiple studies have been reported that disease-associated human single nucleotide polymorphisms (SNPs) were detected. These studies indicated an important factor regarding genetic factors of RA and autoimmune diseases; some of the RA-susceptible polymorphisms also increase the risks of other autoimmune diseases as reported for e.g., STAT4 (ref1) and FCRL3 (ref2) with RA and SLE. Recently, it was also reported that three SNPs, rs509749, rs3817407 and rs1333065 in Ly9 which is one of the SLAM gene family, were associated with systemic lupus erythematosus (SLE). We investigated allele frequencies of 3SNPs in Ly9 using 830 RA patients and 658controls. 3SNPs in Ly9 had no association in a Japanese population (rs509749; P=0.3266, rs3817407; P=0.0746, rs1333065; P=0.8329). Furthermore, we selected 9 tagging-SNPs in Ly9 from the HapMap project uging the method of Gabriel et.al. to cover densely. The most significant SNP was rs4017732 (P=0.0005, OR=0.66) and the others were not significantly different (P>0.01). We performed replication study using independent case and control set, however we could not validate the association of the rs4017732 (P>0.01). 1) Remmers, E.F. et al. N Engl J Med 357, 977-86 (2007). 2) Kochi, Y. et al. Nat Genet 37, 478-85 (2005). 3) de Bakker, P.I. et al. Nat Genet 37, 1217-23 (2005).

A Text-Based Strategy to Identify Common Pathways In Regions Implicated by Association Studies. *S. Raychaudhuri[1,2,3], R. M. Plenge[1,2], M. J. Daly[1,3]* 1) Broad Institute, Cambridge, MA; 2) Division of Rheumatology, Brigham and Women's Hospital, Boston, MA; 3) Center for Human Genetic Research, Massachusetts General Hospital, Boston, MA.

Genome-wide association (GWA) studies for diseases and clinical phenotypes have begun to identify associated variants in multiple regions of the genome. To deliver on the promise that these studies will provide insight into the biological mechanisms of disease however, we need to identify the specific genes and functional pathways implicated by disease-associated variants. One approach is to identify the key relationships between genes from associated regions. Here we describe a statistical method, *Gene Relationships Among Implicated Loci* (GRAIL), which assesses the degree of relatedness between genes in regions implicated by GWA studies using published text within PubMed Abstracts. We apply GRAIL to the results of recent successful GWA meta-analyses: 19 SNPs associated to serum lipid levels and 42 SNPs associated with height. In each case, GRAIL identifies highly significant subsets of related genes within the regions implicated by these SNPs and additionally identifies keywords that describe the relevant functions that significantly link genes from distinct associated regions. We then further test the ability of this text-based method to prioritize regions for replication studies, proposing that those regions that include genes that are functionally related to genes in other regions would be enriched for true positive associations. In examining top 74 results from a recent Crohns disease meta-analysis, GRAIL identifies a subset of regions containing genes with significantly more relationships to genes in other associated regions than expected by chance. These regions were in fact enriched (p = 0.001) for true positive associations in a subsequent replication effort, where the 10 out of 11 highest scoring regions replicate. This demonstrates the clear ability of GRAIL to identify relevant functional connections between novel associated regions in an objective, automated fashion. We will further demonstrate the ability of GRAIL to interpret copy number variant regions in autism.

TNFA -308G>A promoter polymorphism disease severity, but not response to anti-TNF therapy in patients with rheumatoid arthritis. *M. Coenen[1], E. Toonen[1], J. Fransen[2], W. Kievit[2], A. de Brouwer[1], T. Radstake[2], C. de Gendt[3], T. Jansen[4], H. Scheffer[1], P. van Riel[2], P. Barrera[2], B. Franke[1], the DREAM consortium* 1) Department of Human Genetics, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands; 2) Department of Rheumatology, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands; 3) Department of Rheumatology, Rijnstate Hospital, Arnhem, the Netherlands; 4) Department of Rheumatology, Medical Centre Leeuwarden, Leeuwarden, the Netherlands.

Rheumatoid arthritis (RA) is a severe chronic inflammatory disease and genetic factors are known to play an important role in its pathogenesis. Genetic factors are also involved in determining the prognosis of RA and the response of patients to therapy. For the treatment of patients it would be worthwhile if their disease outcome and response to treatment could be predicted at the beginning of the disease. In this way each individual patient could be treated in the most adequate way. We investigated whether the -308G>A promoter polymorphism in the *TNFA* gene is associated with response to anti-TNF treatment and/or disease severity in patients with RA. Two patient samples were genotyped for the *TNFA* -308G>A promoter polymorphism (rs1800629) using TaqMan SNP genotyping. One sample (n=426) consisted of patients from the Dutch Rheumatoid Arthritis Monitoring (DREAM) register, for whom detailed information on their response to anti-TNF therapy was available, the other consisted of patients from a long-term observational early RA inception study (n=208). The -308G>A polymorphism was not associated with anti-TNF response at 3 and 6 months. The GG genotype, and more specifically the G allele, was associated with increased radiographic joint damage over a period up to 9 years after diagnosis, independently of age, gender, rheumatoid factor status and disease activity. Our data show that the suitability of the *TNFA* -308G>A promoter polymorphism as a marker for response to monoclonal anti-TNF antibodies in clinical practice is still questionable, and confirm that the polymorphism is associated with RA disease severity.

Dynamic Modification Strategy of the Israeli Prenatal Carrier Screening Protocol: Inclusion of the Oriental Jewish Group to the Cystic Fibrosis Panel. *O. Reish[1, 2], Z. Borochowitz[3], V. Adir[3], M. Shohat[4], E. Pras[5], A. Storch[6], A. Orr-Urtreger[7], Y. Yaron[7], S. Shalev[8], F. Fares[9], R. Gershoni[10], T. Falik-Zaccai[11], D. Chapman-Shimshoni[1]* 1) Genetics Inst, Assaf Harofeh Medical Ctr, Zerifin, Israel; 2) Sackler School of Medicine, Tel Aviv University, Tel Aviv; 3) Bnai-Zion Medical Center,Haifa; 4) Rabin Medical Center, Petah Tikva; 5) Sheba Medical Center, Tel-Hashomer; 6) Meir Medical Center, Kfar Saba; 7) Tel Aviv Medical Center, Tel Aviv; 8) Ha'emek Hospital, Afula; 9) Carmel Medical Center, Haifa; 10) Rambam Medical Center, Haifa; 11) Western Galilee Hospital, Nahariya.

Purpose: To determine the carrier frequencies of the recently identified mutations in CF patients in Jews of Oriental origin, in Israel. **Methods:** Ten medical centers participated in the study. Three mutations were screened in Iraqi Jews : two splice site mutations - 3121-1G>A and 2751+1insT and one nonsense mutation - the Y1092X. The I1234V a missense mutation within the nuclear binding domain of CFTR gene was screened in Jews of Yemenite origin. **Results:** In 3474 chromosomes derived from Iraqi Jews, the 3121-1G>A ,Y1092X and 2751+1insT mutations had a carrier frequency of 1:68.5 and 1:435 respectively while the third mutation was not detected. In 2072 screened Yemenite chromosomes, the I1234V mutation disclosed a 1:130 carrier frequency. **Conclusion:** The 3 Iraqi mutations add up to an allele frequency of 0.84% which stands within the Israeli Society of Medical Geneticists' inclusion criteria of 1:60 carrier frequency and therefore, prompted the inclusion of Iraqi Jews to the prenatal screen. The 2751+1insT that was detected in patients only, was included in the screening panel to optimize detection rate. The I1234V has not met the criteria for inclusion, but is offered on diagnostic basis and can be added to the screening panel of protocol-recommended-origins when mixed with Yemenite. This study demonstrates the dynamic modifications of the Israeli prenatal CF screening protocol based on newly detected founder mutations confirmed in a large scale population, while taking into account mutation impact and inter-communal admixture.

Association of *GIRK3* gene polymorphisms with methamphetamine and alcohol dependence. *D. Nishizawa[1], J. Hasegawa[1], S. Kasai[1], H. Ujike[2,13], N. Ozaki[3,13], Y. Sekine[4,13], T. Inada[5,13], M. Harano[6,13], T. Komiyama[7,13], M. Yamada[8,13], M. Iyo[9,13], N. Iwata[10,13], I. Sora[1,11,13], S. Higuchi[12], K. Ikeda[1], Japanese Genetics Initiative for Drug Abuse (JGIDA)* 1) Dept Molecular Psychiatry, Tokyo Inst Psychiatry, Tokyo, Japan; 2) Department of Neuropsychiatry, Graduate School of Medicine and Dentistry, Okayama University, Okayama, Japan; 3) Department of Psychiatry, Nagoya University Graduate School of Medicine, Nagoya, Japan; 4) Department of Psychiatry and Neurology, Hamamatsu University School of Medicine, Hamamatsu, Japan; 5) Department of Psychiatry, Seiwa Hospital, Institute of Neuropsychiatry, Tokyo, Japan; 6) Department of Neuropsychiatry, Kurume University School of Medicine, Kurume, Japan; 7) Division of Psychiatry, Iida Hospital, Ritsuzan-Kai med Corp, Iida, Japan; 8) Department of Psychogeriatrics, National Institute of Mental Health, National Center of Neurology and Psychiatry, Tokyo, Japan; 9) Department of Psychiatry, Graduate School of Medicine, Chiba University, Chiba, Japan; 10) Department of Psychiatry, Fujita Health University School of Medicine, Toyoake, Japan; 11) Department of Psychobiology, Tohoku University Graduate School of Medicine, Sendai, Japan; 12) Institute of Clinical Research, National Alcoholism Center Kurihama Hospital, Yokosuka, Japan; 13) Japanese Genetics Initiative for Drug Abuse (JGIDA).

This study investigated whether polymorphisms in the *GIRK3* gene, a candidate gene for rewarding effects, could be indices of risk factors for methamphetamine (METH) and alcohol dependence in Japan. In the association study for five single nucleotide polymorphisms (SNPs), significant difference was found in allele frequency (p=0.0279) for the C1339T single nucleotide polymorphism (SNP) between the METH-dependent and control subjects. Also, significantly higher frequencies were observed in the alcohol-dependent patients for the GTCCG (p=0.0005) and CCTCA (p=0.0346) haplotypes compared to the control subjects. Although the underlying molecular mechanism remains to be elucidated, our data indicate that the *GIRK3* C1339T SNP and haplotypes could serve as markers for predicting the vulnerability to METH or alcohol dependence.

COL5A1 Gene Duplication and Trisomy 9q34 in a Patient with a Complex Ehlers Danlos Syndrome Phenotype.

S. Kallish, J. Keenan, R. E. Schnur Department of Pediatrics, The Children's Regional Hospital at Cooper University Hospital, Camden, NJ.

A 41-year-old woman was referred for evaluation of vascular Ehlers Danlos syndrome (EDS), a diagnosis given to her at age 13. She had a history of developmental delay and congenital heart disease. Connective tissue variations included shoulder dislocations, scoliosis, easy bruising, and poor wound healing.

The patient appeared prematurely aged with numerous atrophic scars. She had translucent skin and easily visible vasculature. She had a positive Gorlin sign, thoracic kyphoscoliosis, arachnodacytyly, and hyperextensibility of the thumbs. Dysmorphic features included maxillary hypoplasia with a tall chin and forehead. Her eyes were closely spaced and deep-set. She had a narrow nasal bridge, a palpably bifid nasal tip, and a high palate with a midline bulge.

The patient was found to have a duplication of the 9q subtelomeric region translocated onto distal 10q. The 10q subtelomere was not deleted. Thus, the patient has a pure trisomy of the 9q subtelomeric region. CGH confirmed partial trisomy 9q34.11q34.3.

Significantly, the *COL5A1* gene maps to this region. *COL5A1* mutations cause classical EDS. Fibroblasts from our patient were assayed for types I and III collagen with normal results. After finding the 9q duplication, the skin biopsy was studied for *COL5A1* RNA expression. All three *COL5A1* alleles were expressed.

COL5A1 duplication of in this patient suggests that this may directly contribute to her features of EDS. Her phenotype is consistent with other reported cases of duplications of 9q and Marfanoid features. One hypothesis is that the overproduction of *COL5A1* protein leads to excess production of 1(V) homotrimers rather than the heterotrimers that comprise normal type V collagen. Diagnostic testing of collagen genes in patients with connective tissue abnormalities should potentially include screens for gene duplications in addition to sequencing and deletion analysis.

mtDNA mutation screening in blood and sperm of infertile oligoasthenozoospermic (OA) men. *S. Venkatesh[1], R. Kumar[1], R. Kumar[2], NP. Gupta[2], RK. Sharma[3], RN. Bamezai[4], R. Dada[1]* 1) Anatomy, Laboratory for Molecular Reproduction and Genetics, New Delhi, India; 2) Department of Urology, AIIMS, New Delhi, India; 3) ART center , Army Research and Referral Hospital, N Delhi; 4) School of life sciences, JNU, New Delhi, India.

Sperm mitochondria are source of energy (ATP) for spermatogenesis and sperm motility by oxidative phosphorylation (OXPHOS) pathway. mtDNA is highly susceptible to free radical mediated damage and also accumulate mutation due to a very basic repair mechanism and lack of protamines. Since the number of mtDNA in male germ cell are very few as compared to somatic cell, we studied the frequency of mutation in blood (somatic) and sperm cells and to also determine which is ideal to study in understanding pathogenesis of OA. The study included 45 infertile patients with 20% progressive motile and 20 million sperm/ml in the semen and 30 age and ethnically matched fertile controls (who have initiated a successful pregnancy in the last 12 months). Semen analysis was performed according to WHO (1999) guidelines. Both blood and sperm DNA were isolated and sequenced for the mitochondrial genes (ND, Cyt b, CO I, and ATPase) by standard PCR-DNA sequencing protocol. More number of nucleotide changes was detected in the sperm mtDNA than the blood mtDNA of the OA patients. An average of 11.1 nucleotide changes were observed in the mtDNA of sperm compared to the mtDNA of the blood (6.6) for 10 nucleotides [7028 (CO I), 8279, 8280, 8701, 8860 (ATPase), 12612, 12705 (ND5), 15043, 15226, 15257(Cyt.b). Based on our results we conclude that frequency of nucleotide changes are more in the germ cells compared to the somatic cells. Thus molecular screening of germ cell mtDNA is a better diagnostic marker than somatic cells to understand the etiology of OA and counsel these men before they opt for ICSI.

A potential common genetic pathway linking depression to cardiovascular disease. *J. Zhao, R. Patel, A. Zafari, V. Vaccarino, A. Quyyumi* Dept Medicine, Emory Univ, Atlanta, GA.

Background: Coronary artery disease (CAD) and depression are prevalent comorbidity of major clinical significance. Genetic factors and inflammation are implicated in both disorders. We hypothesized that genetic variants in inflammatory pathways related to leukotriene metabolism explain the comorbidity of depression with CAD. Methods: Caucasian subjects undergoing coronary angiography at Emory University had genotyping for 12 SNPs in the leukotriene A4 hydrolase gene (LTA4H). CAD was defined as 50% stenosis in 1 epicardial coronary artery or a history of MI. Controls were subjects with completely smooth coronary arteries and no history of MI. Depression was defined as a score 10 in the Patient Health Questionnaire 9. Single marker analysis was assessed by the 2 test. Haplotype associations were tested using likelihood ratio tests. Empirical significance levels were determined by permutation tests. The relationship between genetic variants and CAD or depression was further determined using logistic regression, adjusting for traditional coronary risk factors. Results: The sample for analyses included 1319 angiographic CAD patients and 425 controls (30.4% female). Of these, 198 were depressed. None of the SNPs was individually associated with either CAD or depression. However, a six-SNP haplotype in LTA4H, named HapE, showed a significant protective effect on both CAD (OR = 0.55, 95% CI [0.37-0.83], p = 0.005) and depression, independent of traditional risk factors including age, sex, smoking, history of diabetes and hypertension. The protective effect of HapE on depression was only observed in women (OR = 0.28, 95% CI [0.11-0.72], p = 0.008), but not in men (OR = 0.98, 95% CI [0.56-1.72], p = 0.94). The prevalence of HapE in the study population was ~ 14%. Conclusion: HapE protects against both CAD and depression. It explains 6% of the comorbidity of depression with CAD. Our finding provides the first evidence that genetic variants in leukotrienes may underlie the link between depression and CVD, and may be implicated in the gender difference in depression. Replication and functional studies are needed to test this hypothesis.

In vitro modulation of TCF7L2 gene expression in human pancreatic cells. *K. Khalooghi[1], S. Hashemi[2], N. Mehraban[1], P. Amiri[1], J. Tavakkoly Bazzaz[1], B. Larijani[1], MM. Amoli[1]* 1) Endocrinology and metabolism research centre, Tehran university, medical sciences; 2) Department of genetics, Faculty of Medicine, Tehran university, medical sciences.

Background: Several studies have recently reported strong association between type 2 diabetes and variation in the transcription factor 7-like 2 (TCF7L2) gene, which has been confirmed by several other genome-wide studies. However the physiological implications of this transcription factor on the pathogenesis of type 2 diabetes is not yet known. Aim: The aim of this study was to investigate the alteration in TCF7L2 gene expression in human pancreatic cell line in response to various factors in vitro. Methods: MIA Paca-2 cell line (Human Pancreas cell line) was cultured in the presence of curcumin, lipopolysaccaride and Glucose (low and high concentration). TCF7L2 gene expression was determined using quantitative real-time RT-PCR. Results: Treatment with curcumin significantly increased TCF7L2 gene expression to 3.24 fold (1.7-log fold) (p 0.003) compared to the controls while treatment with LPS decreased TCF7L2 gene expression to 0.88 -fold (-0.18-log). On the other hand, glucose increased TCF7L2 gene expression in pancreatic cell line. Conclusion: Our data suggest a role for TCF7L2 in glucose homeostasis. The contrary effect of curcumin and LPS on expression of TCF7L2 in pancreatic cells supports a role for TCF7L2 in their survival and function in inflammatory conditions.

Familial interstitial Xq27.3q28 duplication encompassing the FMR1 gene: a "contre-type" of Fragile X syndrome? *M. Rio[1], V. Malan[1,2], A. Toutain[3], JM. Lapierre[1], S. Gobin[1,2], G. Royer[1], JP. Bonnefont[1,2], A. Munnich[1,2], M. Vekemans[1,2], L. Colleaux[1,2]* 1) Département de génétique, Unité INSERM 781, Hôpital Necker-Enfants Malades, Paris, France; 2) Université Paris Descartes, Paris France; 3) Service de génétique, hôpital Bretonneau, Tours, France.

X-linked mental retardation is a very common disorder which accounts for 5-10% of cases of retardation in males. Fragile X syndrome is one of the most common inherited form resulting from loss of expression of the FMR1 gene. Partial duplication of the long arm of the X chromosome is uncommon. It leads to functional disomy of the corresponding genes and has been reported in males with mental retardation. Using1 Mb array comparative genomic hybridization (array CGH), we identified a small interstitial Xq27.3q28 duplication encompassing the FMR1 gene in a large X linked syndromic mental retardation family. The two males, initially referred for genetics work up because of mental retardation, share a similar clinical phenotype including bilateral testicular ectopia, small stature and minor facial dysmorphism. They also have phenotypic features caused by testosterone deficiency: gynecomastia, sparse body hair, high pitched voice and small testicles. The duplication was confirmed by fluorescence in situ hybridization and segregates with the disease in the families. The three carrier females were all asymptomatic and have a skewed X inactivation pattern. We suggest that Xq27.3q28 duplication results in a novel clinically recognizable syndrome. Interestingly, clinical features observed in our patients are opposite of those observed in fragile X syndrome patients such as macroorchidism and tall stature. Although additional patients will be necessary to further delineate this condition, our data already suggest that increased FMR1 gene copy number may be responsible for the phenotype in our patients. Fine molecular characterization of this duplication is presently underway to determine other genes that might contribute to the clinical features observed in these patients.

Mapping Short Reads to the Human Genome. *S. Katzman[1], D. Haussler[1,2,3]* 1) Department of Biomolecular Engineering, Univ California, Santa Cruz, Santa Cruz, CA; 2) Center for Biomolecular Science and Engineering, Santa Cruz, CA; 3) Howard Hughes Medical Institute, Santa Cruz, CA.

Ultra high throughput DNA sequencing (UHTS) technologies are characterized by low cost per base sequenced, but have read lengths much shorter than the 500-1000bp reads from Sanger sequencing technology. Although UHTS promises inexpensive whole genome human resequencing, mapping its 25, 35, or 50bp reads to the human reference presents a difficult challenge. In the presence of errors and polymorphism, policies for mapping a read must ensure a high level of accuracy. Here we characterize the mappability of the human genome by checking for matches of nmers from the reference sequence to anywhere else in the reference. Single reads, even of high quality, that are shorter than 30bp will leave a significant portion of the human genome unmapped. Above that length there is a gradual improvement. But paired-end reads of sufficient length, even of 30mers, may provide a way to escape shorter islands of unmappability in the human genome. Still, we demonstrate that false mapping from regions not represented in the reference may be a serious problem. It may lead to an unacceptably high rate of false SNPs, getting worse rather than better with increasing depth of coverage.

Implementation of the JaundiceChip as a Clinical Diagnostic Tool. *C. Liu[1], B. Richardson[1], A. Schalk[1], R. Mourya[2], J. A. Bezerra[2], K. Zhang[1]* 1) Human Genetics, Cincinnati Children's, Cincinnati, OH; 2) Gastroenterology, Cincinnati Children's, Cincinnati, OH.

Background: Inheritable neonatal intrahepatic cholestasis is one of the leading liver diseases in children. Mutations in ATP8B1, ABCB11, ABCB4, JAG1 and SERPINA1 have been detected in patients with different types of the disease. Identification of the mutations in these patients confirms specific diagnosis and guides personalized treatment. However, due to the lack of hot spots, sequencing has been used to identify the disease causing mutations, which is very time consuming and costly. Method: To address this issue, a resequencing microarray, JaundiceChip, was developed as a research tool to sequence the coding regions of these five genes. To translate the technology into a clinical diagnostic setting, new protocol and analysis algorithm were developed and validated to improve the sensitivity and specificity of the assay. Results: Thirty samples were tested using an updated fragmentation and hybridization protocol based on Affymetrixs recommendation. As a result, the average call rate reached 98.7%, a big increase from 93.5% as previously shown. To further increase the sensitivity of the chip, no call positions from 30 chips were plotted and two major groups with distinct patterns were identified. The first group contains 108 positions that are fixed in every sample tested, corresponding to 46% of no call positions. Three of them overlap with reported missense mutations in JAG1 gene. The second large group contains 10% of no call positions, which are unique to only one of the samples tested and called out in the other 29 samples. New analysis algorithm was developed to read out these positions and identified three missense mutations and one small deletion. All mutations reported by GSEQ software were subjected to direct confirmational sequencing and 100% accuracy was achieved. Conclusion: The re-sequencing based JaundiceChip provide a new tool for molecular diagnosis of inherited cholestatic liver diseases with high sensitivity and specificity.

Frequency of the Copy Number Variants Affecting the *OTC* Locus in Patients with OTC Deficiency. *O. A. Shchelochkov[1], L. Y. Tang[1], A. Pursley[1], F. Li[1], M. Geraghty[2], U. Lichter-Konecki[3], P. M. Fernhoff[4], S. Copeand[5], T. Reimschisel[6], S. Cederbaum[7], B. Lee[1], A. C. Chinault[1], L. J. Wong[1]* 1) Dept Mol & Hum Genetics, Baylor Col Medicine, Houston, TX; 2) Dept of Genetics, Childrens Hospital Eastern Ontario, Canada; 3) Childrens National Medical Center, Washington, D.C; 4) Dept of Hum Genetics, Emory University, Decatur, GA; 5) Dept of Pediatrics, University of Iowa, Iowa City, IA; 6) Div of Med Genetics, Washington University School of Medicine, St. Louis, MO; 7) UCLA Medical Center, Los Angeles, CA.

Ornithine transcarbamylase (OTC) deficiency is an X-linked inborn error of metabolism with the estimated prevalence of 1:14,000. Previously reported data suggest that only approximately 80% of OTC deficiency (OTCD) patients have an identifiable mutation by sequencing the *OTC* gene.

To elucidate the molecular etiology in patients with clinical signs of OTCD and negative *OTC* sequencing, we performed array comparative genomic hybridization (aCGH) using a custom-designed targeted 44k oligonucleotide array.

DNA samples from a total of 56 OTCD patients were analyzed. Thirty nine patients (39/56 or 69.6%) were found to have disease-causing point mutations in the *OTC* gene. The remaining 17 patients (17/56 or 30.4%) showed normal sequencing results or failure to amplify all or part of the OTC gene. Among those patients, eight (8/56 or 14.3%) were found to have deletions ranging from 24 kb to 10.6 Mb, all involving the *OTC* gene. Nine OTCD patients (9/56 or 16.1%) had normal sequencing and oligoarray results. Analysis of the deletions did not reveal shared breakpoints, suggesting that non-homologous end joining or replication-based mechanism might be responsible for the formation of the observed rearrangements.

In summary, we demonstrated that approximately half of the patients with negative *OTC* sequencing may have *OTC* gene deletions readily identifiable by the targeted oligonucleotide-based aCGH. Thus, aCGH should be considered in *OTC* sequencing-negative patients with classic symptoms of the disease. Further studies are necessary to elucidate the molecular mechanisms of observed rearrangements.

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Preimplantation Genetic Diagnosis for Nonsyndromic Deafness by polar body and blastomere biopsy. *G. Altarescu, T. Eldar-Geva, B. Brooks, E. Haran-Zylber, E. J. Margalioth, E. Levy-Lahad, P. Renbaum* Zohar PGD Lab & IVF Unit, Shaare Zedek Medical Ctr, Jerusalem, Israel.

Objective: 1- To develop an efficient and reliable universal protocol for PGD of nonsyndromic deafness; 2- assess heterozygosity rates of polar body 1 (PB1), marker informativity and Allele Drop Out rates (ADO) for polar body and blastomere PGD. Design and Methods: Polar bodies 1 and 2 (PB1 and PB2) and blastomere biopsy was performed by mechanical drilling. Three mutations GJB2 35delG, 167delT and GJB6 delD13S1830 and the markers: D13S141, D13S175, D13S633, D13S1275, D13S250, D13S232, GJB2-AT1, GJB2-AT2, GJB2-TG1, GJB2-TG2, GJB2-AC1, were used to set up a universal multiplex PCR protocol to be used for PGD in non syndromic deafness. Results were only assigned for samples with at least three informative flanking markers (including the familial mutation). Results: Eight couples underwent 19 PGD cycles resulting in a pregnancy rate of 21% and delivery of 3 unaffected children. Six cycles (32%) were performed by polar body biopsy, 6 by blastomere biopsy, 6 by frozen blastomere biopsy and in one cycle by both PB and blastomere biopsies. In 17 cycles (90%) at least 2 embryos were transferred and in one cycle one embryo was transferred. The rate of successful diagnosis for blastomere PGD was 91% and for PB PGD, 87%. Only 11 of 68 PB1s (17%) were heterozygote. Similar ADO rates (19%) were observed for both heterozygote PB1s and blastomeres. While all families had at least two informative markers on each side of the gene for PB PGD, none had 4 fully informative markers for blastomere PGD. Conclusions: We have developed a universal single cell multiplex protocol for nonsyndromic deafness with a high efficiency of diagnosis for PGD. Although PB PGD allows more informative marker assessment, most PB1 are homozygous, and ADO rates were seen to be similar, therefore, blastomere biopsy appears to be the method of choice for this autosomal recessive disease.

Molecular Characterization of Ataxia Oculomotor Apraxia in Saudi Arabia. *D. M. Bakheet[1], N. A. Al Tassan[1], L. J. Al Sharif[1], D. S. Khalil[1], T. S. Al Khairallah[2], S. A. Bohlega[2]* 1) Department of Genetics, King Faisal Specialist Hospital & Research Centre, Riyadh 11211, Saudi Arabia; 2) Department of Neurosciences, King Faisal Specialist Hospital & Research Centre, Riyadh 11211, Saudi Arabia.

Background: Ataxia with Oculomotor Apraxia (AOA) is a rare autosomal recessive disorder with two subtypes. AOA1 presents with cerebellar ataxia and oculomotor apraxia between 2 and 18 years of age and is accompanied later in life by sensory-motor neuropathy which may be associated with choreathetosis or mental retardation, hypoalbuminemia, hypercholesterolemia, normal immuno-globins and alpha-fetoprotein levels. Patients with AOA2 present with gait ataxia, cerebellar atrophy, sensory- motor neuropathy, ocular-motor aparaxia, elevated immuno-globins and alpha-fetoprotein levels with a later age of onset (10-22 years). Although the two forms are not quite distinctive phenotypically,two different genes (*APTX* and *SETX*) on chromosome 9 have been linked to AOA. **Objective:** This study aims to screen for mutations in patients with AOA. **Methods**: Five Saudi families with a clear diagnosis of either AOA1 or AOA2(2 or more affected individuals) have been identified and enrolled in this study. Comprehensive screening for the whole open reading frame (ORF) of genes of interest were performed. **Results:** A novel truncating mutation (c.6859 C>T, R2287X) in the *SETX* gene was identified in one family with AOA2, while the other families with either AOA1 or AOA2 have been negative for mutations in both genes. Analysis of *MRE11* gene, which is known to be implicated in ataxia like (AT-like) disorder, identified a common reported mutation W210C in exon 7 in two families with AOA1 phenotype. The remaining families were negative for mutations in the ORF of all 3 genes highlighting allelic or genetic heterogeneity of these disorders. Investigation of this heterogeneity is underway through screening of non-coding regions of these 3 genes, and also genome-wide linkage analysis. **Conclusion:** These results demonstrate the genetic diversity of this disorder and indicate the possible involvement of more genes in the development of this disease.

DNA copy number variations in major psychosis. *J. Tang[1,3], L. Cheng[1], D. Craig[2], M. Josephson[2], S. Christian[1], E. Gershon[1], X. Chen[3], C. Liu[1]* 1) Departments of Psychiatry and Human Genetics, University of Chicago, Chicago, IL; 2) The Translational Genomic Research Institute, Phoenix, AZ; 3) Institute of Mental Health, The Second Xiangya Hospital, Central South University, Changsha, Hunan, P.R. China.

In order to identify copy number variations (CNVs) in schizophrenia, bipolar disorder and major depression, and to test correlations between CNVs and these diseases, we analyzed CNVs in postmortem brain DNA from individuals with DSM-IV diagnosed SZ (50), BD (49), MD(15) and matched controls (49),provided by the Stanley Medical Research Institute Brain - Array and Consortium Collections. The Affymetrix 5.0 SNP array was used for genotyping. Partek software was used to obtain estimates of CNVs. Copy number baseline was created from pools of all control samples,and the intensity of each samples data was normalized to the copy number baseline. CNVs were detected using a Hidden Markov Model, with a region defined as consistent variation in at least 3 adjacent probes. Secondary analyses of the genomic DNA hybridization data were performed, on allele signal ratio and loss of heterozygosity (LOH), which can give support information on CNVs. 2502 CNVs (from 1226 loci) were detected from autosomes. 22 CNVs (from 20 loci) were detected from X chromosome. The average number of CNVs detected per individual was 15.5. Of 2524 CNVs, 1148 CNVs were heterozygous deletions, 562 CNVs were homozygous deletions, and 814 CNVs were duplications. The median size of CNVs was 31kb. Three large CNVs on chromosome 9 were detected in schizophrenia and bipolar diseases. We compared the CNVs identified in our analysis to those present in the Database of Genomic Variants. 156 CNVs (96 loci) in our data were novel CNVs. Of 2524 CNVs, 906 CNVs are singleton CNVs, and 1618 CNVs were non-singleton CNVs. In tests for association for 43 common CNVs, CNV-304 has marginal significant P value (p=0.086) after Bonferroni correction. We further investigated genes that were deleted or duplicated exclusively in patients. 35 genes in CNVs were recorded exclusively in patients with major psychosis:6 in bipolar disorder,8 in major depression and 21 in schizophrenia.

Functional Impact of Polymorphisms in the Promoter Region of the Apoptosis Gene MDM2. *M.-E. Lalonde[1], D. Sinnett[1,2]* 1) CHU Ste-Justine, University of Montreal, Montreal, Quebec, Canada; 2) Department of Pediatrics, University of Montreal, Montreal, Quebec, Canada.

Introduction: MDM2 is involved in the intrinsic apoptosis pathway. Thus deregulations in the expression levels of the MDM2 gene could lead to an accumulation of cells (due to a lack of apoptosis), creating a favourable environment for genetic instability, oncogene activation and tumorigenesis. Furthermore, the MDM2 promoter region contains regulatory polymorphisms (rSNPs) that could influence its expression and modify its transcription levels. Materials and Methods: The initial search for rSNPs in the MDM2 proximal promoter was done using the dbSNP database. Seven rSNPs were selected for genotyping in 40 individuals of European descent. We then tested the impact of the three most frequent promoter haplotypes on expression levels using a luciferase gene reporter assay in 4 different cell lines (HeLa, HepG2, JEG3 and Jurkat). Gel shift experiments were also carried out to test the impact of the promoter rSNPs on nuclear protein binding to putative transcription factors binding sites. Results and Discussion: Following genotyping we identified 3 common promoter haplotypes characterised by a combination of 4 rSNPs. Gene reporter assays revealed allelic differences in MDM2 promoter activity: haplotype -1494G-1208_-1169del -182G was shown to increase the expression level 5-fold when compared to the promoter haplotype -1494G-1208_-1169nondel -182C. Furthermore, the gel shift experiments showed differential DNA-protein binding at these different positions. Conclusion: These promoter rSNPs/haplotypes are associated with variable MDM2 expression levels at least in vitro. Because of the highly allele regulated nature of apoptosis, such allelic imbalance may contribute to tumorigenesis. Acknowledgements: This project was supported by Genome Canada/ Québec and the Quebec Optimist Clubs.

The ESR2 gene polymorphism is associated with increased risk of high tension glaucoma in women. *F. Mabuchi[1], Y. Sakurada[1], K. Kashiwagi[1], Z. Yamagata[2], H. IIjima[1], S. Tsukahara[1]* 1) Dept Ophthalmology, Univ Yamanashi, Chuo, Japan; 2) Dept Health Sciences, Univ Yamanashi, Chuo, Japan.

Purpose: To assess whether genetic polymorphisms of estrogen receptor beta (ESR2) are associated with primary open angle glaucoma. Methods: Japanese patients with normal tension glaucoma (NTG, n = 213), and high tension glaucoma (HTG, n = 212), and 191 control subjects were analyzed for the ESR2 gene polymorphisms (rs1256031 and rs4986938) using allele specific primer PCR technique, and genotypic and allelic frequency differences between NTG, HTG patients and control subjects were estimated. The mean age at the time of blood sampling was 63.7 13.6 years (SD) in patients with NTG, 62.9 14.8 years in patients with HTG, and 65.7 11.4 years in the control subjects. Results: Although no statistically significant differences of the genotype and allele frequencies were found out between the NTG patients and the control subjects, there were significant differences in the genotype frequencies of rs1256031 and rs4986938 between the HTG patients and control subjects in women (P = 0.033 and P = 0.043 respectively, Chi-square test). The frequencies of the C allele of rs1256031 and G allele of rs4986938 were significantly higher in patients with HTG compared to the control subjects in women (rs1256031: 53.6% vs. 43.4%, P = 0.044; rs4986938: 89.2% vs. 80.6%, P = 0.027, Fishers exact test). Conclusion: The ESR2 gene polymorphisms are associated with HTG, and may be used as a marker for this disease association in women.

Prenatal diagnosis of Holt-Oram syndrome through ultrasonography and molecular analysis. *S. M. Weiss, C. L. Yates* Northwestern University, Chicago, IL.

Holt-Oram syndrome (HOS) is an autosomal dominant disorder characterized by upper-extremity malformations, a personal or family history of congenital heart defects, and an increased risk for cardiac conduction disease. Upper-extremity malformations involve the radial, thenar or carpal bone(s). Most common cardiac defects include atrial and ventricular septal defects. Approximately 70% of individuals who present with clinical criteria have an identifiable mutation in the TBX5 gene [12q24.1]. TBX5 encodes for a transcription factor that plays a role in cardiac septation and limb development. Approximately 85% of TBX5 gene mutations are de novo. Prenatal diagnosis of low risk pregnancies is difficult due to the variable expressivity of the gene mutations and the overlap of clinical features with numerous other conditions. We report on an affected fetus ascertained through routine ultrasound in a G2P1001 woman with an unremarkable family history. Unilateral absence of the radius and a possible heart defect were identified on level II ultrasound at 19w2d. Subsequent fetal echocardiogram suggested atrial septal defect (ASD). Amniocentesis revealed a normal 46, XX karyotype. Testing on amniocytes for a mutation in the TBX5 gene revealed a heterozygous G>T nucleotide substitution in exon 4 (D111Y), resulting in the replacement of an aspartic acid with a tyrosine at amino acid position 111 of the TBX5 protein product. Prompt diagnosis through TBX5 gene mutation analysis allowed for accurate genetic counseling and expectant management.

Genetic Structure Analysis of an East African Population. *M. Luo[1], J. Sainsbury[1], G. Vandomselaar[3], T. Ball[1], J. Kimani[2], F. Plummer[1,3]* 1) Dept Medical Microbiology, Univ Manitoba, Winnipeg, MB, Canada; 2) University of Nairobi, Nairobi, Kenya; 3) National Microbiology Laboratory, Winnipeg, MB, Canada.

As the cradle of humanity, East Africa contains the most genetically diverse population owing to early settlements and migrations. The population has also been under extensive selective pressure by various infectious diseases. Genetic epidemiology and disease association studies require a clear understanding of existing population genetic substructure to avoid spurious associations. We conducted population substructure analysis of a population of women enrolled in the Pumwani Sexworker cohort in Nairobi, Kenya using three different approaches. HLA class I (A, B, C) were genotyped and haplotypes of more than 800 women were analyzed using arlequin 3.11; 444 polymorphic SNPs randomly selected from 23 chromosomes from 432 individuals enrolled in the Pumwani Sexworker cohort were analyzed and compared with SNPs data from three HapMap populations, CEU (European), YRI (African) and CHB+JPN (Asian) using Structure 2.2; and Principle component analysis (PCA) of 423,688 SNPs with HelixTree(v6.3.6, Golden Helix). These analyses showed that there is no significant population substructure in this East African population. As expected this East African population co-clusters with the African YRI population, but is genetically more diverse than the YRI population sample included in the HapMap study.

Let-7d: A regulator of epithelial-mesenchymal transition (EMT) and idiopathic pulmonary fibrosis (IPF). *K. Pandit[1], D. Corcoran[1], H. Yousef[1], D. Handley[1], A. Ben-Yehudah[1], A. Pardo[2], M. Selman[2], O. Eickelberg[3], M. Yarlagadda[1], P. Ray[1], P. Benos[1], N. Kaminski[1]* 1) University of Pittsburgh, USA; 2) Instituto Nacional de Enfermedades Respiratorias, Mexico; 3) University of Giessen Lung Center, Germany.

Introduction: IPF is a chronic, lethal lung disease resulting in death within 3-5 years of diagnosis. TGF- plays a central role in the pathogenesis of IPF, with SMAD3 being its effector molecule. In this study, we investigated the effect of TGF- on the microRNA, let-7d and the role of this microRNA in the pathogenesis of IPF. **Methods and Results:** Discriminative motif analysis of upstream regions of intergenic microRNAs recognized a SMAD3 binding site in the promoter of let-7d. Electrophoretic mobility shift assay and SMAD3 chromatin immunoprecipitation were performed to confirm the let-7d/SMAD3 binding. Stimulation of A549 cells with recombinant TGF-, led to a decrease in let-7d ($p<0.05$) 6h later and a corresponding increase in HMGA2 ($p<0.05$) as quantified by qRT-PCR. HMGA2 is a key regulator of the TGF--induced EMT known to be expressed only during embrogenesis and carcinogenesis. Transfection of A549 cells with a let-7d inhibitor resulted in an increase in HMGA2 and markers of EMT including N-cadherin, vimentin and -smooth muscle actin, by qRT-PCR and immunofluorescence. Let-7d is downregulated in IPF lungs ($p<0.05$) compared to control lungs by qRT-PCR. In control lungs, let-7d was localized in the bronchial and alveolar epithelial cells by in situ hybridization. HMGA2, a target of let-7d, is increased 12-fold in IPF lungs by qRT-PCR and localizes to alveolar epithelial cells by immunohistochemistry. Ongoing experiments include inhibition of let-7d in the lungs of mice by intratracheal administration of a chemically-modified oligonucleotide to study fibrotic changes in the lung tissue. **Conclusion:** Our results suggest that let-7d is under direct transcriptional regulation of TGF- and is a key mediator of EMT. Thus, let-7d occupies a strategic place in the TGF- signaling pathway; its downregulation in IPF suggests that it plays a major role in the pathogenesis of this devastating disease.

Maternal uniparental disomy 14 spectrum and differential diagnosis of Prader-Willi syndrome. *S. Saitoh[1], K. Hosoki[1], M. Kagami[2], T. Ogata[2]* 1) Dept Pediatrics, Hokkaido Univ Sch Medicine, Sapporo, Japan; 2) Dept Endocrinology and Metabolism, National Research Institute for Child Health and Development, Tokyo, Japan.

Maternal uniparental disomy 14 [upd(14)mat] is characterized by intrauterine growth retardation, neonatal hypotonia, small hands and feet, a characteristic facies including prominent forehead and precocious puberty. These phenotypes of upd(14)mat resemble those of Prader-Willi syndrome (PWS), in particular during the infancy. In our series of molecular investigation of patients who were initially suspected to have PWS, we have identified five patients with upd(14)mat and one patient with an epimutation of chromosome 14q32.2. All patients were initially identified by a DNA methylation test for the *MEG3* gene, and subsequent microsatellite polymorphism studies confirmed the parental origin of each chromosome 14 allele. In the patients with upd(14)mat, 3 patients had full upd(14)mat with a normal karyotype, 1 patient had mosaic upd(14)mat with a normal karyotype, and 1 patient had full upd(14)mat with a mosaic supernumerary marker of chromosome 14 origin. The patient with epimutation of 14q32.2 was thoroughly investigated for microdeletion, segmental upd, and DNA methylation status in 14q32.2, and microdeletion and segmental upd were ruled out. The IG-DMR, which is the proposed primary imprinting site, and the *MEG3* promoter were hypomethylated, indicating a maternal epigenotype on the paternally derived chromosome 14. The clinical features of these 6 patients were basically consistent with those of reported phenotypes for upd(14)mat, including pre- and postnatal growth retardation, although the patient with an epimutation had a normal birth weight. Patients with the upd(14)mat phenotype who have a small deletion of paternal origin in 14q32.2 have been reported (Kagami et al, Nat Genet 40:237-42, 2008). Therefore, it is now clear that the upd(14)mat phenotype is caused not only by upd(14)mat but by epimutation or small deletions.

The rise and fall of mtDNA mutations in cultured single cells. *Y.-G. Yao[1, 3, 4], S. Kajigaya[1], X. Feng[1], L. Samsel[2], J. P. McCoy[2], N. S. Young[1]* 1) Hematol Branch, NHLBI, NIH, USA; 2) Flow Cytometry Core Facil, NHLBI, NIH, USA; 3) Key Lab Anim Models & Human Dis Mech, Kunming Inst Zool, Kunming, Yunnan, China; 4) State Key Lab Genet Res & Evolut, Kunming Inst Zool, Chinese Acad Sci, Kunming, Yunnan, China.

We have described a marked level of mtDNA sequence variation in single hematopoietic cells from healthy human donors and leukemia patients. To test whether single cells can quickly acquire mtDNA mutations and lead to this mutation pattern, we compared mtDNA sequence variation among single CD34+ cells, single colonies grown from CD34+ cells, and single cells from a colony grown from a CD34+ cell after one-week culture. Single cells from a Jurkat T cell line culture, a subclone of Jurkat cell established from one cell after one-month culture, and single cells from a colony of the subclone culture were analyzed to discern potential effects of long- and short-term culture. Single CD34+ cells harbored more mutations than did single colonies grown from CD34+ cells from the same donor. Colonies from adults had higher levels of point mutations than did those from cord blood. The proportion of cells harboring mutations varied within different colonies and did not present a uniformly homogeneous pattern. Proliferation of a single cell harboring certain acquired mutation(s) could cause a change of the main haplotype in the expanded cell population. These results provide new insights into the rise and fall of mtDNA mutations in cultured hematopoietic cells and caution their causal role in the aging of hematopoietic stem cells.

Genome-wide association scan of tag SNPs for congenital heart defect. *J. J. Kim[1], K. J. Kim[2], I. S. Park[2, 3], J. K. Lee[1, 2]* 1) Asan Institute for Life Sciences, University of Ulsan College of Medicine, Seoul, Korea; 2) Genome Research Center for Birth Defects and Genetic Disorders, Asan Medical Center, Seoul, Korea; 3) Department of Pediatrics, University of Ulsan College of Medicine, Asan Medical Center, Seoul, Korea.

Congenital heart defects (CHDs) are the most common developmental anomaly, affecting approximately 1% of all live births and the leading cause of death in newborns. Despite the many advances in our understanding of cardiac development, the etiology of CHDs is only partly illuminated. Numerous epidemiologic studies have established the heritable nature of CHDs. However, very few genes causing CHDs have been identified so far. Our aim was to identify genetic variants involved in the risk of CHDs. A genome-wide association scan with the Illumina HumanHap300-Duo array was performed in 13 familial cases of CHDs and 90 normal controls. The overall genotype call rate was 99.9%. Allelic comparison was made between cases and controls for 276,644 single nucleotide polymorphisms (SNPs). Thirty six SNPs were statistically significant after Bonferroni correction. The most strongly associated SNP ($P = 4.78^{10-11}$) was rs3856852 at 5 flanking region of the CRBN gene on chromosome 3p26.3. We found several possible significant regions harboring biologically meaningful genes associated with heart development. For example, the significant rs801521 SNP was located in the intron region of the HDAC9 gene encoding histone deacetylase 9, which has been reported to play a role in heart development. Another significant SNP, rs2157727 was located in 5 flanking region of the CLDN5 gene that is commonly deleted in patient with velocardiofacial syndrome. Further replication studies are needed to confirm these association results and identify real causative SNPs for CHDs.

***CDKL5* disruption by t(X;18) in a girl with West syndrome.** *A. Nishimura[1,2], T. Takano[3], T. Mizuguchi[1], H. Saitsu[1], Y. Takeuchi[3], N. Matsumoto[1,2]* 1) Department of Human Genetics, Yokohama City University of Medicine, Yokohama, Japan; 2) The Yokohama City University 21st Century Center of Excellence (COE) Program of MEXT; 3) Department of Pediatrics, Shiga University of Medical Science, Otsu, Shiga, Japan.

Breakpoint sequences of the balanced translocation t(X;18)(p22;p11.2) found in a girl with West syndrome were determined. Der(X) breakpoint lost three nucleotide with two nucleotide with unknown origin added, and der(8) never lose or add any nucleotides. *CDKL5* at Xp22 was found to be disrupted in the intron 17. This is the third case of West syndrome due to CDKL5 disruption by the chromosomal translocation.

An Epistatic Model for Characterizing the Genetic Control to Complex Diseases. *T. Liu[1], A. Thalamuthu[1], JJ. Liu[1], RL. Wu[3], C. Chen[2]* 1) Genome Institute of Singapore, Singapore; 2) Dept. of Pharmacology, National University of Singapore & Dept. of Medicine, National University Hospital, Singapore; 3) Department of Statistics, Genetics Institute, University of Florida, Gainesville, FL.

Interactions within and between different genes, coined the epistasis, have been increasingly recognized to be of paramount importance in the pathogenesis of most common human diseases, such as cancer or cardiovascular disease, and patients' responsiveness to a medicine. The most common approaches for detecting genome-wide epistasis are based on genetic mapping that associates phenotypic variation of a trait with a linkage map constructed by polymorphic markers. Integrating the principle of quantitative genetics, we here propose a computational model for dissecting a complex disease into its genetic action and interaction components composed of causal single nucleotide polymorphisms (SNPs) in a simple case-control association study. The model is also extended to infer disease-associated haplotypes and used to probe concrete nucleotoxic sites that contribute to variation in a complex disease with a simple case-control design, and thus provide a way to push epistasis identification at the DNA sequence level.We formulated a mixture-model framework to compute the frequencies of haplotypes through the EM algorithm, and test the differences of the occurrence of haplotype combination that leads to epistasis of various kinds between the case and control groups. For each kind of epistasis, the 2 statistics was derived from a two by two contingency table based on combined haplotype frequencies between the case and control groups. Computer simulations show that the method is more powerful and informative than existing approaches. Testing the new model on a stroke candidate-gene case-control data set, we identify several within-gene and between-gene interactions from different candidate genes that trigger significant interaction effects on stroke.

X-linked female-limited epilepsy and cognitive impairment caused by protocadherin 19 mutations. *L. M. Dibbens[1], P. S. Tarpey[2], K. Hynes[1], M. A. Bayly[1], I. E. Scheffer[3], D. H. Geschwind[4], S. McKee[5], S. F. Berkovic[3], M. R. Stratton[2], J. C. Mulley[1], J. Gecz[1]* 1) Dept Genetic Medicine, Women's & Children's Hosp, North Adelaide, Australia; 2) Wellcome Trust Sanger Institute, Hinxton, United Kingdom; 3) Epilepsy Research Centre and Department of Medicine , University of Melbourne, Victoria, Australia; 4) Neurology Department and Semel Institute for Neuroscience and Behaviour , University of California at Los Angeles, Los Angeles, California, USA; 5) Northern Ireland Regional Genetics Service, Belfast City Hospital, Belfast, Northern Ireland, UK.

Epilepsy and Mental Retardation limited to Females (EFMR) is an X-linked disorder with an unusual pattern of sex-limited expression. Human disorders arising from mutations on the X chromosome are typically characterized by affected males and unaffected carrier females. In contrast, EFMR spares transmitting males and affects only carrier females. EFMR is characterized by early onset seizures in previously normal infants, followed by developmental regression of varying severity. Five new EFMR families were ascertained on the basis of their inheritance pattern. Haplotype analysis of the five families was consistent with gene localization on Xq22. Aided by systematic re-sequencing of 737 X chromosome genes we identified mutations in the Protocadherin 19 (PCDH19) gene in seven EFMR families. Five mutations result in the introduction of a premature termination codon. Study of two of these demonstrated nonsense mediated decay of mRNA. The two missense mutations are predicted to affect adhesiveness of PCDH19 through impaired calcium binding. Murine and human brain expression analyses of PCDH19 support an important role in brain function. These results identify protocadherins as a new gene family directly associated with epilepsy and brain cognition. We are now investigating the role of PCDH19 in other related neurological conditions.

CD14 -550C/T polymorphism modifies the effect of daycare attendance on total and specific IgE levels in children. *S. Hattori[1], Y. Mashimo[1], M. Funamizu[1], N. Shimojo[2], Y. Okamoto[3], Y. Kohno[2], A. Hata[1], Y. Suzuki[1]* 1) Department of Public Health; 2) Department of Pediatrics; 3) Department of Otolaryngology, Chiba University Graduate School of Medicine, Chiba, Japan.

Although results of studies examining a relation between daycare attendance and development of asthma have been conflicting, a consistent inverse relation between daycare attendance and atopy or serum IgE levels has been reported. CD14 is a pattern recognition molecule for bacterial endotoxin and involved in the Toll-like receptor pathways. Polymorphisms in the CD14 gene have been shown to modify effect of environmental factors such as endotoxin on the development of atopy and allergic diseases. Daycare attendance is thought to increase chance of infection. If frequent exposure to endotoxin is one of the mechanisms of protective effect of daycare attendance against atopy, a CD14 polymorphism may modify this daycare effect. Four hundreds and seventy-three school children with 6 to 12 years of age were asked whether they had attended daycare in the first 2 years of life. We determined total and 8 specific IgE levels and genotyped CD14 -550C/T in 410 children. Effects of the gene, daycare, and their interactions on the serum IgE levels were evaluated with generalized linear models. Atopy (one or more positive specific IgE) was evaluated with logistic regression models. Daycare attendance was associated with lower total IgE levels in the entire study population (P=0.022). In children with CC genotype, daycare showed little effects on the total IgE value (log(total IgE): daycare(-): 1.980.76; daycare(+): 1.880.77, P=0.54). In children with CT or TT genotype, daycare significantly lowered the total IgE level (daycare(-): 2.090.63, daycare(+): 1.580.51, P=0.000097). The interaction between daycare attendance and the polymorphism was significant for both total IgE (P=0.0034) and mite-specific IgE (P=0.00023). This interaction was also significant for atopy (P=0.0075). The effect of daycare attendance on the IgE levels and atopy was modified by CD14 -550C/T genotype. The mechanism that daycare attendance affects atopic status probably involves innate immune responses to microorganisms.

Unique 2, 17 autosomal translocation in an infertile man. *M. Kumar[1], S. Venkatesh[1], D. Pathak[1], RK. Sharma[2], R. Dada[1]* 1) Lab. for Molecular Reproduction and Genetics, Department of Anatomy, All India Institute of Medical Sciences, New Delhi, India; 2) ART center, Army Research and Referral Hospital, New Delhi.

Chromosomal aberrations are one of the most common genetic causes associated with male infertility. In spite Y chromosomal defect attribute to impaired spermatogenesis, autosomes also play an important role in regulating spermatogenesis. Here we report a novel translocation involving chromosome 2 and chromosome17 in a 34 years old infertile man with severe oligozoospermia. Pedigree showed no family history of infertility. 72 hours blood culture was set up, GTG banding was done, and chromosomes were classified according to ISCN guidelines. Repeated semen analysis showed highly viscous and incomplete liquefaction even after 30 minutes with sperm count 1 million/ml. Peripheral karyotype analysis revealed 46, XY t(2;17) (qter;q12) chromosome complement. To the best of our knowledge such autosomal translocation in q arm of chromosomes 2 and 17, has not been previously reported. Though several studies have been shown role of sex chromosomes in the germ cell production, autosomes also play an important role in regulation of spermatogenesis. Such autosomal translocation result in severely impaired spermatogenesis and manifest as oligozoospermia. Thus a large number of cases with autosomal structure abnormalities need to be studied to establish genotype and phenotype correlation and to understand the role of autosomal genes in germ cell development and differentiation. Additional study of cases parent genotype, fluorescent in situ hybridization and molecular screening is necessary to characterize the gene involved in translocation, position effect and its impact on regulation of germ cell development.

A Comprehensive Evaluation of SNP Genotype Imputation. *D. Ellinghaus[1], M. Nothnagel[2], S. Schreiber[1,3], M. Krawczak[2,3], A. Franke[1]* 1) Institute for Clinical Molecular Biology, Kiel, Germany; 2) Institute of Medical Informatics and Statistics, Christian-Albrechts-University, Kiel, Germany; 3) PopGen Biobank, Christian-Albrechts-University, Kiel, Germany.

Genome-wide association studies have contributed significantly to the genetic dissection of complex diseases. In order to increase the power of existing marker sets further, methods have been proposed to predict individual genotypes at untyped loci from other marker sets by imputation, usually employing HapMap as a reference population. Although various imputation algorithms have been used in practise already, a comprehensive evaluation of these approaches, using genome-wide SNP data from one and the same population, is still lacking. We therefore investigated three genotype imputation programs (IMPUTE, MACH, and PLINK) using data from 449 German individuals genotyped in our laboratory for three different, commercially available genome-wide SNP arrays (Affymetrix 5.0 [500k], Affymetrix 6.0 [1000k], and Illumina 550k). Since the same 449 DNA samples were genotyped on all arrays, and since the arrays contained only partially overlapping marker sets, extensive genome-wide benchmarking using varied confidence thresholds became possible through a comparison of the imputed and observed genotypes derived with the different arrays. We observed that HapMap-based imputation in a European population is powerful, accurate and reliable, even in highly variable genomic regions such as the extended MHC on chromosome 6p21. However, while genotype predictions were found to be highly accurate for all three programs, the number of SNPs for which imputation was actually carried out varied substantially.

Significance of cytokines and ICAM as susceptibility genes for end stage renal disease. *G. Tripathi[1], P. Ranganath[2], S. N. Sankhwar[1], R. K. Sharma[3], S. Agrawal[2]* 1) Urology, CSM Medical University, Lucknow, Uttar Pradesh, India-226003; 2) Department of Medical Genetics, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow -226014; 3) Department of Nephrology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow -226014.

Objectives Cytokines and intercellular adhesive molecules (ICAM) play crucial role in different immunopathological conditions and hence may be responsible for pathogenesis of primary kidney disease and progression to end stage renal disease (ESRD). We have made an attempt to explore the role of different polymorphisms of these cytokines and ICAM genes as a susceptibility factors for ESRD. **Methods** The distribution of cytokine and adhesion molecule gene polymorphisms was analyzed in 258 ESRD patients and ethnically matched 569 controls. Individuals were genotyped for IL-4 (C590T), IL-6 (G174C), TNF- (G308A and G238A) and ICAM -1 (A469G) gene polymorphisms using standard PCR-RFLP based method. **Results** There was a significant difference between ESRD patients and control groups both at the biochemical parameters and at genotypic level. Significant difference was observed in genotype frequencies of the TNF- -308AA (p=0.001, OR=7.61, 95%CI=2.1-27.9), TNF- -238AA (p=0.001, OR=5.8, 95%CI=2.2-15.1). Further C allele of IL-6 -G174C and G allele of ICAM-1 A469G were significantly different in ESRD when compared to controls (p=0.0001; OR=5.5, 95%CI=3.9-7.7 and p0.0001; OR=3.8, 95%CI=3.1-4.7) respectively. For the IL-4 C590T polymorphism, though the homozygous mutant genotype (TT) was not found to be significantly associated with ESRD, a statistically significant association with T allele (p=0.0001) was found with the ESRD. Further, combined analysis revealed a higher risk in ESRD patients with low IL-4 and high IL-6 producing genotypes and high producing genotype of TNF- (308 and 238) with the increase risk of ~6.0 fold and 3.3 fold respectively. The haplotype analysis of TNF- (308 and 238) reveled high risk genotype with the risk ~8.5 fold (p=0.034). **Conclusions** Our results suggest that IL-6, IL-4, TNF- and ICAM gene polymorphism may be a risk factor for ESRD.

Differential Downregulation of Gastrin by IL1B Promoter Polymorphism through Signalling Intermediates NFKB and SMAD7. *D. Datta De[1], M. Maitra[2], S. Bhattacharjya[1], A. Choudhury[3], G. K. Dhali[3], S. Roychoudhury[1]* 1) Molecular and Human Genetics Department, Indian Institute Of Chemical Biology, Kolkata, West Bengal, India; 2) Department of Pediatrics and Molecular Biology, UT Southwestern Medical Center, Dallas, Texas; 3) Departments of Medicine and Gastroenterology, Institute of Postgraduate Medicine and Experimental Research, Kolkata-700 020, India.

More than half of the worlds population is chronically infected by Helicobacter pylori. IL1B promoter polymorphisms -511 C>T and -31C>T have been associated with Helicobacter pylori mediated gastro-duodenal diseases. Gastrin,a gastric acid-modulating hormone,is involved in the pathogenesis of gastro-duodenal ulcerations.The present study investigated the effect of IL1B promoter polymorphism on the transcriptional activity of the human gastrin promoter in a gastric epithelial cell line, AGS,and analyzed the underlying molecular mechanisms.Treatment of AGS with IL1B resulted in a 20-fold reduction in gastrin expression.Detailed investigation revealed that IL1B represses gastrin through TAK1, TAB1 that ultimately up regulates NFkB. A 40% release of IL1B mediated gastrin repression,as a result of inhibition of NFkB translocation,further suggested the presence of NFkB independent pathway.It was observed that IL1B up regulates Smad7, which inhibits gastrin expression by 6 folds.These results were also validated in vivo, where, at least a 3 fold lower expression of Smad7 and NFkB in H. pylori infected individual with ulcer compared to infected asymptomatic individuals was observed. NCOR and HDAC1 were also involved in repression of gastrin by IL1B.A 3-fold increase in IL1B expression was observed when AGS cells were transfected with -31TIL1B expression construct in comparison to -31CIL1B.This differential effect of IL1B promoter variants on its transcription subsequently translated in a 2 fold greater repression of gastrin by -31TIL1B. The signaling intermediate NFkB and Smad7 also revealed higher expression in the -31TIL1B transfected AGS cells. Experiments with HDAC inhibitor also suggested that at a particular dose, the repression on the gastrin promoter in -31CIL1B-transfected cells was greater than in cells transfected with -31TIL1B.

First evidence for an association of a functional variant in the miRNA-510 target site of the serotonin receptor type 3E gene with diarrhea predominant irritable bowel syndrome. *J. Kapeller[1], L. A. Houghton[2], H. Mönnikes[3], J. Walstab[4], D. Möller[1], H. Bönisch[4], B. Burwinkel[5], F. Autschbach[6], B. Funke[6], F. Lasitschka[6], N. Gassler[7], C. Fischer[8], P. J. Whorwell[2], W. Atkinson[2], C. Fell[2], K. J. Büchner[9], M. Schmidtmann[3], A.-S. Wisser[3], G. Rappold[1], B. Niesler[1]* 1) Dept. of Human Molecular Genetics, University of Heidelberg, Germany; 2) Neurogastroenterology Unit, University of Manchester, Wythenshawe Hospital, UK; 3) Dept. of Medicine, Inst. of Neurogastroenterology, Martin-Luther Hospital Berlin, Germany; 4) Inst. of Pharmacology and Toxicology, University of Bonn, Germany; 5) Molecular Epidemiology, DKFZ Heidelberg, Germany; 6) Inst. of Pathology, University of Heidelberg, Germany; 7) Inst. of Pathology, RWTH Aachen, Germany; 8) Inst. of Human Genetics, University of Heidelberg, Germany; 9) Div. of Hepatology, Gastroenterology and Endocrinology; Charité Berlin; Germany.

Diarrhea predominant irritable bowel syndrome (IBS-D) is a complex disorder related to dysfunctions in the serotonergic system. As cis-regulatory variants can play a role in the etiology of complex conditions, we investigated the untranslated regions (UTRs) of the serotonin receptor type 3 subunit genes *HTR3A* and *HTR3E*. Mutation analysis was carried out in a pilot sample of 200 IBS patients and 100 healthy controls from the United Kingdom. The novel *HTR3E* 3'UTR variant c.*76G>A (rs62625044) was associated with female IBS-D (P = 0.033, OR = 8.53). This association was confirmed in a replication study including 119 IBS-D patients and 195 controls from Germany (P = 0.0046, OR = 4.92). Pooled analysis resulted in a highly significant association of c.*76G>A with female IBS-D (P = 0.0002, OR = 5.39). In a reporter assay, c.*76G>A affected binding of miR-510 to the *HTR3E* 3'UTR and caused elevated luciferase expression. *HTR3E* and miR-510 co-localize in enterocytes of the gut epithelium as shown by *in situ* hybridization and RT-PCR. This is the first example indicating microRNA related expression regulation of a serotonin receptor gene with a cis-regulatory variant affecting this regulation and appearing to be associated with female IBS-D.

Short Tandem Repeat Typing Technologies used in Diagnosis Testing. *M.Ali. Saremi*[1,2] 1) Kawsar Genomic Research Center, Tehran, Tehran, Iran; 2) Baqiyatallah Medical Sciences University - Human Genetics Research Center, Tehran, Iran.

Micro satellite DNA loci or short tandem repeats (STRs) are abundant in eukaryotic genomes and are often used for diagnosis testing of closely related populations or species. These diagnosis testing are usually constructed by using some genetic distance measure based on allele frequency data, and there are many distance measures that have been proposed for this purpose. In the past the efficiencies of these distance measures in diagnosis testing have been studied mathematically or by computer. Because short tandem repeat (STR) loci are highly polymorphic, they are very useful for studying the evolutionary relationships of closely related populations or species. However, a number of statistical problems should be solved before this approach can be used effectively. Five ml of blood samples were obtained from the cases and their parents, in 7 ml falcon tubes. They were subsequently divided into two tubes, one for the stock and next uses and the other containing ethylene (EDTA) anticoagulant for use in PCR analysis. For DNA analysis, standard RGDE DNA extraction procedure was used to extract DNA from collected blood samples. PCR amplification was performed using primer sequences for five STR markers. Primer sequences were obtained from the Genome Database (http://www.gdb.org/). These markers are polymorphic and have recently been used for molecular diagnosis and parental origin determination in other population. The results of the fluorescent PCR analysis are shown effective application of STR markers for diagnosis of genetic diseases. The estimate of the recombination frequency in a population and precise mapping of the recombination events are critical for genetic counseling and prenatal diagnosis. This analysis can be easily performed thanks to an automated, fast, and accurate method based on the separation by capillary gel electrophoresis of the fluorescent-labeled amplified alleles of STRs that span the entire etiologic genetic factors. I am grateful to Saremi Mahnaz and Maghsoudi Sahar from Kawsar Genome Research Center for revising and editing the text.

Immunogenetics of systemic sclerosis: new clues for pathophysiology and related pulmonary fibrosis. *Y. Allanore[1], P. Dieudé[2], M. Guedj[2], J. Wipff[1], A. Munnich[1], C. Boileau[1]* 1) INSERM U 781, Necker Hosp, Paris, France; 2) CHU Bichat, Rheumatology, Paris, France.

Systemic sclerosis (SSc) is a severe orphan connective tissue disease with no proven effective drug. Our aim is to unravel the genetic factors underlying SSc which belongs to complex diseases. Some data suggest that the autoimmune component of SSc may be shared with that seen in other complex immune diseases, with micro-array studies suggesting a type I interferon signature. Therefore we tested the hypothesis that the PTPN22 and IRF5 (a regulator of interferon alpha production) genes, both involved in various autoimmune diseases, are associated with SSc. We tested DNA from individuals of European French Caucasian origin. SSc patients with the co-occurrence of auto-immune diseases associated with PTPN22 or IRF5 were excluded prior to the respective analyses. 659 SSc patients and 504 controls were genotyped for 7 PTPN22 SNPs including rs2476601 (1858C>T). The IRF5 rs2004640 (G>T) functional SNP was genotyped in a discovery set (427 SSc patients and 380 controls) and in an independent replication set (454 SSc patients and other 380 controls). No association was detected between the 7 PTPN22 SNPs tested and SSc. Haplotype analysis revealed a strong association between a risk haplotype carrying the 1858T allele (P = 1.52x10-7) and SSc. The meta-analysis of the available data (1772 SSc patients and 1262 controls) provided further evidence that PTPN22 rs2476601 confers susceptibility to SSc, particularly in the anti-topoisomerase I positive patients. Association between the IRF5 rs2004640 TT genotype and SSc was found in both discovery and replication sets. In combined populations, the TT genotype frequency was significantly increased in SSc patients compared to controls (P=0.002, OR 1.58, 95%CI 1.18-2.11). An association was observed in the sub-group of patients with positive antinuclear antibodies (Pcorr=0.04, OR 1.59 [1.16-2.17]) and fibrosing alveolitis (Pcorr=0.001, OR 2.07 [1.38-3.11]). No interaction between PTPN22 and IRF5 SNPs was detected. These data provide new insight into the pathogenesis of SSc, including clues to the mechanisms leading to specific disease subtypes.

Genetic contribution to all cancers: the first demonstration using the model of breast cancers from Poland stratified by age at diagnosis and tumor pathology. *J. Lubinski[1], M. Korzeń[2], B. Górski[1], C. Cybulski[1], T. Dębniak[1], A. Jakubowska[1], K. Jaworska[1], D. Wokolorczyk[1], K. Mędrek[1], J. Matyjasik[1], T. Huzarski[1], T. Byrski[1], J. Gronwald[1], B. Masojć[1], M. Lener[1], A. Szymańska[1], J. Szymańska-Pasternak[1], P. Serrano Fernandez[1], S. Narod[3], R. Scott[4]* 1) Intl Hereditary Cancer Ctr, Pomeranian Med Univ, Szczecin, Poland; 2) Technical Univ of Szczecin, Szczecin, Poland; 3) Centre for Research on Womens Health, Univ of Toronto, Toronto, Ontario, Canada; 4) Discipline of Medical Genetic, Univ of Newcastle, and the Hunter Medical Research Inst, Newcastle, Australia.

The aim of the study is to verify the hypothesis that genetic polymorphisms are associated with the predisposition to all malignancies. Using as a model breast cancers from the homogenous Polish population (West Pomeranian region) after stratification of 977 patients by age at diagnosis (under 51 years and above 50 years) and by tumour pathology (ductal cancers - low and high grade, lobular cancers, ER-positive/negative) we tested this hypothesis. Altogether 20 different groups of breast cancer cases have been analyzed. The results were compared to a group of unaffected controls matched by age, sex, ethnicity and geographical location and originated from families without cancers of any site among relatives. Molecular alterations selected for analyses included those which have been previously recognized as being associated with breast cancer predisposition. Statistically significant differences between the breast cancer cases and controls were observed in 19 of the 20 analyzed groups. Genetic changes were present in more than 90% of the breast cancer patients in 18 of 20 groups. The highest proportion of cases with constitutional changes - 99.3% (139/140) was observed for lobular cancers. The number and type of genetic marker and/or the level of their association with the specific cancer predisposition was different between groups. Markers associated with majority of groups included: BRCA1, CHEK2, p53, TNRnTT, FGFRnAA, XPD CC/AA and XPD GG. Some markers appeared to be group specific and included polymorphisms in CDKN2A, CYP1B1, M3K nAA, and RS67.

Genomewide Association Study in Ankylosing Spondylitis Identifies Major non-MHC Genetic Determinants of Disease Susceptibility. *D. Evans[1], P. Leo[2], A. Sims[2], W. Maksymowych[3], M. Ward[4], M. Stone[5], P. Rahman[6], M. Weisman[7], R. Inman[8], D. Gladman[8], J. Davis[9], T. Learch[7], L. Savage[10], L. Diekman[11], P. Danoy[2], J. Pointon[12], X. Zhou[11], P. Wordsworth[12], J. Reveille[11], M. Brown[2,12]* 1) Univ of Bristol, Bristol, UK; 2) Univ of Queensland, Brisbane, Australia; 3) Univ of Alberta, Edmonton, Canada; 4) Intramural Research Program, NIAMS, Bethesda, MD; 5) RNHRD, Bath, UK; 6) Memorial Univ, St John's,Canada; 7) Cedars-Sinai Med Ctr, Los Angeles, CA; 8) Toronto Western Hospital, Toronto, Canada; 9) UCSF, San Francisco, CA; 10) Spondylitis Assn of America, Sherman Oaks, CA; 11) Univ of Texas-Houston HSC, Houston, TX; 12) Univ of Oxford, Oxford, UK.

Ankylosing spondylitis (AS) is an inflammatory arthritis, which can lead to fusion of the spine and other affected joints. In order to identify genetic variants predisposing to AS, we enrolled Australian (n=69), British (n=1016) and North American (n=983) cases of European descent fulfilling modified New York Criteria. Control genotypes were obtained from the 1958 British Birth Cohort(n=1500) and from the Illumina iControlDB database (n=3434). Cases were genotyped for 317K SNPs using the Illumina HumHap300 SNP chip. Analysis was performed using Eigensoft to correct for population substructure. The well-known MHC association with AS was confirmed, as were associations with ARTS-1, IL23R and around IL-1R2/R1. Two regions not previously reported achieved genome-wide significance,on chromosome 2p15(p=1.1x10-14) and chromosome 21q22(p=2.6x10-10). 21 unique regions achieved suggestive significance, including SNPs in the gene TNFR1(p=4.8x10-6),over expression of which causes sacroiliitis in mice,and near TRADDD, which interacts with TNFR1(p=3.2x10-5). A confirmation study of these findings is near completion. Finally, we computed likelihood ratios to determine the diagnostic potential of screening individuals for these genetic variants. For many genotypic combinations, the positive and negative predictive value of genetic tests surpassed other diagnostic instruments including MRI scanning. The high prognostic value of genetic tests for AS suggests that they may be of use in screening in both clinical and population settings.

Analysis of matrix metalloproteinase-2 gene polymorphisms in high myopia. *E. Hsi, H. Juo* Graduate Institute of Medical Genetics, Kaohsiung Medical University, Kaohsiung, Taiwan.

Purpose: The sclera contains approximately 90 percent collagen. The matrix metalloproteinase-2 (MMP2) is responsible for degradation of scleral extracellular matrix, which may contribute to myopia. This study was aimed to systemically investigate the polymorphisms at MMP2 and their relationship to high myopia.
Methods: A total of 470 cases and 618 controls were included in the study. A case was defined as a refraction -6 D and control -1.5 D. Seventeen tagging single nucleotide polymorphisms (tSNPs) were genotyped. Statistical analyses included Hardy-Weinberg equilibrium (HWE) test, chi-squared test to evaluate the genotypic effect, linkage disequilibrium (LD) estimation, LD block construction and haplotype analysis. Subset analysis was also conducted for cases with highly myopic parents. The Bonferroni correction was used to correct for multiple testing.
Results: One SNP was not in HWE and thus was removed for further analysis. Single marker analyses did not find any SNP with a significant p value after correcting for multiple testing. There were five haplotype blocks and the fourth block yielded an overall p value of 3×10^{-5} in our first batch of sample (n=1006), and 2×10^{-3} the replication sample (n=710).
Conclusions: This is the first large human study to comprehensively study the tSNPs at the MMP2 gene for high myopia. Our study indicated that a rare causal allele is in strong LD with the fourth block, which showed a significant protective effect against high myopia.

Altered brain gene expression profiles associated with the pathogenesis of phenylketonuria in a mouse model. *J. W. Park, E. S. Park, E. N. Choi, H. Y. Park, S. C. Jung* Dept Biochemistry, School of Medicine, Ewha Womans Univ, Seoul, Korea.

Phenylketonuria (PKU) is an autosomal recessive disorder caused by a deficiency of phenylalanine hydroxylase (PAH), which catalyzes the conversion of phenylalanine to tyrosine. The resultant hyperphenylalaninemia causes mental retardation, seizure, and abnormalities in behavior and movement. Gene expression profiling of brain tissue from a mouse model of PKU revealed overexpression of transthyretin (Ttr), sclerostin domain containing 1 (Sostdc1), alpha-Klotho (Kl), prolactin receptor (Prlr), and early growth response 2 (Egr2). The upregulated state of these genes in brain tissue was confirmed using real-time PCR and Western blotting. In contrast to its overexpression in the brain, TTR expression was low in the sera of PKU mice offered unrestricted access to a diet containing phenylalanine. Expression of TTR decreased in a time-dependent manner in 0.9 mM phenylalanine-treated HepG2 cells and in 1.2 mM phenylpyruvate-treated HepG2 cells. In contrast, expression of TTR increased in 0.5 mM phenyllactate-treated HepG2 cells. The modification of TTR gene expression by phenylalanine, phenylpyruvate and phenyllacate could be mediated through Ttr promoter region. These findings indicate that Ttr and other genes play important roles in the pathogenesis of PKU and that phenylalanine and its metabolites, phenylpyruvate and phenyllactate might have a direct effect on the level of TTR in serum.

Alteration of gene expression related to carbohydrate metabolism in placenta of fetus with intrauterine growth restriction. *M. H. Lee[1], Y. J. Jeon[1], Y. J. Kim[2], S. C. Jung[1]* 1) Dept Biochem, School of Medicine, Ewha Womans Universtiy, Seoul, Korea; 2) Dept Obstet and Gynecol, School of Medicine, Ewha Womans Universtiy, Seoul, Korea.

Intrauterine growth restriction (IUGR) is caused by malnutrition of placenta that carries nutrient and oxygen to the fetus. IUGR is suggested to have important consequences for adult health by increasing the risks of metabolic disorder such as hypertension, diabetes, and obesity. The aim of this study was to investigate whether IUGR is associated metabolic disease. Here, we used cDNA microarray analysis to identify differentially expressed metabolic genes in placentas of fetus with IUGR compared with placentas of the normal control group. The expression of genes related to carbohydrate metabolism was up-regulated in placentas of fetus with IUGR including dihydrolipoamide S-acetyltransferase (E2 component of pyruvate dehydrogenase complex, DLAT), 6-phosphofructo-2-kinase/fructose-2, 6-biphosphatase 2, lactate dehydrogenase C, IGFII, and Insulin. In contrast to the result, IGFI was down-regulated in placentas of fetus with IUGR. In the IUGR group, the glucose concentration was significantly decreased in maternal serum and in cord blood. However insulin concentration was increased in maternal serum and in cord blood. Moreover IGFII concentration was higher in maternal serum and lower in cord blood. These results suggest that alteration of carbohydrate metabolism in fetus with IUGR might be important for metabolic disorder cause after birth.

Searching for the natural RNA substrates of DAZAP1, a testis-abundant hnRNP protein. *P. Yen, C. Y. Chung, C. L. Hsu, F. P. Hsiao* Inst Biomedical Sci, Academia Sinica, Taipei, Taiwan.

Deleted in Azoospermia Associated Protein 1 (DAZAP1) was originally identified through its interaction with DAZ, a spermatogenic factor encoded by the human Y chromosome. It is an hnRNP protein that is expressed most abundantly in the testis. It shows a dynamic expression pattern during mouse spermatogenesis, and has the ability to shuttle between the nucleus and the cytoplasm in somatic cells. In addition, it is excluded from the transcriptionally inactive XY bodies in pachytene spermatocytes. These properties suggest a role of DAZAP1 in RNA transcription and transport. The phenotypes of *Dazap1* mutant mice that manifested both infertility and growth retardation indicates that DAZAP1 is required for spermatogenesis as well as normal mouse development. The natural RNA substrates of DAZAP1 remain elusive, though Hori et al. previously showed that DAZAP1 selectively binds in vitro to RNAs containing two consensus sequences AAAUAG and GU1-3AG in separate loop structures. In an attempt to identify DAZAP1s RNA substrates, we used the SNAAP (isolation of specific nucleic acids associated with proteins) technique to immunoprecipitate DAZAP1 associated RNAs from mouse testis extracts using an anti-DAZAP1 antibody. Subsequent differential display using 8 random primers identified several transcripts that were selectively pulled down by the anti-DAZAP1 antibody. Real-time PCR quantification showed that the levels of these transcripts were much lower in the testes of Dazap1 mutant mice than those of the wild-type mice. Additional experiments will be performed to verify the binding of DAZAP1 to these transcripts and to elucidate how DAZAP1 regulates their expression.

Exploration of genes related to X-linked mental retardation (XLMR) by in-house X-tiling array. *S. Honda[1], S. Hayashi[1], I. Imoto[1], I. Inoue[2], T. Yabe[3], K. Tokunaga[4], E. Nakagawa[5], Y. Goto[6], J. Inazawa[1]* 1) Dept Mol Cytogenet, Med Res Inst, Tokyo med & dent Univ, Tokyo, Japan; 2) Div Mol Life Sci, Sch Med, Tokai Univ, Isehara, Japan; 3) Tokyo Metropolitan Red Cross Blood Cent, Tokyo, Japan; 4) Dept Hum Genet, Grad Sch Med, Univ Tokyo, Tokyo, Japan; 5) Div Child Neurol Musashi Hosp, Natl Center Neurol & Psychiat, Tokyo, Japan; 6) Dept MentalRetardation and Birth Defect Res, Natl Inst Neurosci, Natl Cent Neurol & Psychiat, Tokyo, Japan.

An estimated 10-12% of mental retardation (MR) is caused by mutation on the chromosome X. Although 82 X-linked mental retardation (XLMR) genes have been identified to date, many XLMR genes remain to be identified. Known XLMR genes have been identified by conventional positional-cloning strategies, but cryptic chromosome copy number aberrations (CNAs) cannot be detected by routine karyotyping due to its limited resolution, thus we constructed a high-density chromosome X array (MCG X-tiling array), which contains a total of 1001 bacterial artificial chromosome (BACs) throughout chromosome X except pseudoautosomal regions, to identify novel XLMR-associated genes. We have screened 115 families with XLMR or probable XLMR by array-CGH using MCG X-tiling array, and detected CNAs related to MR in 7 families (6%). Among these 7 families, 3 had CNAs in regions involved in known XLMR-associated genes, whereas, other 4 families had CNAs involved in genomic materials which have never been reported. Among them, 2 families had identical CNAs at Xp: arr cgh Xp22.2×2, Xp21.3×2, although those two families are not consanguineous. The CNAs showed complicated recombination and involvement of a part of genes, suggesting that the recombination may be relevant to MR. To investigate the genomic recombination, we carried out inverse-PCR and identified that the recombination breakpoint with CNAs. We next performed genomic PCR which can detect the recombination breakpoint to screen the recombination in the general population from different ethnic group, suggesting that it is possible that this recombination was occurred in Mongol and introduced into Japan.

XY/XX chimerism presenting as hemihypertrophy with pigmentary abnormalities in a male with normal sexual differentiation. *L. Van Maldergem[1 and 2], M.-F. Portnoi[3], M. Gerard-Blanluet[1], M. Cadot[4], S. Lemerle[4]* 1) Clinical Genetics Unit, Centre hospitalier intercommunal, Creteil, Ile-de-France, France; 2) Centre de génétique humaine, Université de Liège, Liège, Belgium; 3) Service de génétique et embryologie médicales, CHU Armand-Trousseau, Paris, France; 4) Service de pédiatrie, centre hospitalier intercommunal, Creteil, France.

A boy born to non-consanguineous parents presented with marked left body hypertrophy, including asymmetric skull and enlarged left limbs. He developed scoliosis. Facial appearence was striking for irides heterochromia, bushy eyebrows and ocular proptosis. Areas of brownish skin hyperpigmentation on left inferior abdominal quadrant, left arm and left leg were present from infancy. His clinical course indicated a stunted growth (-4SD) for which he received growth hormone therapy. His puberty at 13y resulted in fully developed male external genitalia. At 15y, his height was 151 cm(3rd centile). His intelligence is normal. Although karyotype was 46, XY on leukocytes, due to pigmentary abnormalities, we decided to test the hypothesis of a chromosomal mosaicism and evaluated karyotype on cultured fibroblasts grown after skin biopsies taken from hyper- and normo-pigmented areas. A 46,XY/ 46,XX chimerism was evidenced:94/6 cells in hyperpigmented area and 96/4 cells in normal skin region after FISH study. Chimerism results from fusion of different zygotes in the same embryo. Thus, presence of two cell lines resulting from assembly of two fertilized eggs are present in the same individual. It is an exceptional phenomenon with 31 cases being reported until now. Within this group, XY/XX chimerism represents only a few cases and is usually diagnosed on the basis of aberrant blood group typing or abnormal sexual differentiation. Chimerism escapes diagnosis whenever different cell lines harbour the same gender and when aberrant blood group typing is missed. To the best of our knowledge, only one XY/XX patient with normal sex differentiation has been reported until now(Lipsker et al.2008). Here we provide evidence that hemihypertrophy, short stature and irides heterochromia also belong to the clinical spectrum of this exceptional tetragametic phenotype.

Candidate genes controlling longevity in Koreans. *J. W. Park[1,2], D. H. Kim[1,2], Y. Jee[2], H. Y. Cho[2], S. C. Park[3], J. B. Park[1,2]* 1) Dept. Molecular and Cellular Biology, Sungkyunkwan Univ., School of Medicine, Suwon, Korea; 2) Ctr. Genome Research, Samsung Biomedical Research Inst., Seoul, Korea; 3) Dept. Biochemistry, School of Medicine, Seoul National Univ., Seoul, Korea.

Long-lived people may have a unique genetic makeup which is more resistant to the prevalent age-related diseases such as diabetes mellitus, cardiovascular disease, and cancer than the general population. We performed a high-throughput candidate gene study on a genome-wide scale to identify susceptibility variants controlling longevity using 137 nonagenarians/centenarians and 213 young healthy controls ascertained from the Korean Centenarian Study. Among 1,536 SNPs genotyped, we evaluated 486 informative markers located in 179 genes in a series of different association analyses under five genetic models. A total of 60 genes among 179 candidate genes yielded nominal significance in either of allelic or genotypic ^{2} - test. Particularly, six genes (i.e. ADCY3, EGFR, PAX4, PPP1R1A, ALDH2, and CDK10) attracted much attention as potent candidate genes affecting longevity. Among eleven genes consistently yielded significant evidence for allelic, genotypic and haplotypic analyses (i.e. HK2, ERBB4, PCSK1, ITK, EGFR, PAX4, LPL, LYN ADCY9, CDK10, and PPP1R16B), PCSK1 ($p = 0.008$), EGFR ($p = 0.003$), PAX4 ($p = 0.008$), and LYN ($p = 0.002$) remained significant even after the Bonferroni correction for multiple testing. In two way interaction analyses, six, one, three, and two pairs of loci for each of four age-gender groups yielded statistical significance. The results from different statistical analyses consistently supported that the presence of the gene variants might confer susceptibility to longevity in Koreans. While the generalizability of the finding is uncertain, genes identified here may lay the foundations for further studies.

The Study of Mitochondrial A3243G Mutation in Different Tissues. *Y. MA[1], Y. QI[1], F. FANG[2], Y. YANG[3], Y. ZHANG[1], S. WANG[1], P. PEI[1], Y. XU[1]* 1) Central laboratory, Peking University First Hospital, Beijing, China; 2) Department of Pediatrics,Bejing Childrens Hospital, Beijing, 100045, China; 3) Department of Pediatrics, Peking University First Hospital, Beijing, 100034, China.

The most frequent syndromic manifestation of A3243G mutation is the mitochondrial encephalomyopathy, lactic acidosis and stokelike episodes syndrome (MELAS). The A3243G mutation level was higher and more stable in DNA isolated from muscle than that from blood, however, patients often reject the invasive muscle biopsy, and only take mutation analysis in blood, which can reduce the positive mutation level detection, particularly in the oligosymptomatic patients and the asymptomatic relatives. Thus A3243G mutation analysis in blood was not a good choice. Therefore, we compared the A3243G mutation level in five easy accessible tissues of MELAS syndrome patients carrying the A3243G mutation and their maternal relatives, to find the most sensitive tissue, and establish the best and non-invasive inspection method.We studied 33 MELAS patients known to harbor A3243G mutation and their 50 maternal relatives. 18 maternal relatives show oligosymptomatic, and 32 relatives manifest asymptomatic. Total DNA was extracted from the peripheral blood, urine, hair folic, saliva and muscle tissue of the patients and relatives. A3243G mutation was detected by PCR-RFLP method. A3243G mutations were detected in all tissues of the 33 MELAS patients. A3243G mutation ratio in urine was significantly higher than that of blood (t=-11.13, P0.0001), and was similar to that of muscle tissue. A3243G mutations were detected in one tissue or all tissues of the 44 maternal relatives. There were 9 relatives in whom the mutation was not detected in blood while it was clearly present in urine. A3243G mutation ratio in their urine were significantly higher than that of blood (t=-6.87, P0.0001). We observed a close correlation between the level of A3243G mutation in blood and saliva for many patients and relatives (Pearsons correlation coefficient, r=0.651, p=0.001). These data strongly support urine is a good tissue of choice for the diagnosis of A3243G mutation, and the level of A3243G mutation in saliva can represent that in blood.

C2ORF34, A new methyltransferase gene deleted in patients with the 2P21 deletion syndrome. *R. Parvari[1,2], S. Buriakovsky[1,2]* 1) Dept Genetics & Virology, Ben Gurion Univ, Beer Sheva, Israel; 2) National Institute of Biotechnology Negev, Ben Gurion Univ, Beer Sheva, Israel.

The 2p21 deletion syndrome is a unique recessively inherited syndrome presented by an extended Bedouin family. The clinical manifestations of the syndrome were cystinuria, mental and growth retardation, hypotonia, facial dysmorphism and reduced activity of mitochondrial encoded respiratory chain enzymatic complexes. Our previous study revealed the molecular basis of this syndrome as a homozygous deletion of 179,311 bp on chromosome 2p21, and thus was subsequently named the 2p21 deletion syndrome. Further studies to define the transcription content of this interval showed that it includes 4 protein coding genes: type I cystinuria SLC3A1, phosphatase 2C and two uncharacterized genes KIAA0436 and C2orf34. The C2orf34 gene has its first exon in the deletion and thus is not expressed in patients. C2orf34 is highly conserved during evolution and ubiquitously expressed including the tissues affected by the syndrome. We present evidence for its function as a methyltransferase. We hypothesize that the absence of this gene in the patients may have a major contribution to the clinical presentation of the patients.

Successful treatment of spinocerebellar ataxia 6 by systems therapy with medicinal herbs. *T. Okabe* Dept Integrated Traditional Medicine, University of Tokoy Graduate School of Medicine, Hongo, Tokyo, Japan.

The spinocerebellar ataxias (SCAs) are clinically and genetically a heterogeneous group of neurodegenerative disorders. At present,we have no effective therapeutic tools. We report here two cases of spinocerebellar ataxia 6(SCA6)with typical symptoms, which were improved after administration of a mixture of traditional medicinal herbs. A 60-year-old Japanese female suffered from gait disturbance, ataxia and dizziness. Head MRI revealed a typical atrophic image in cerebellum. Genetic tests revealed an expanded allele of 22 CAG repeats at the spinocerebellar ataxia type 6 locus. She was diagnosed as SCA6. A mixture of 18 medicinal herbs (modified Chinkan-sokufu-to) was given according to the differential diagnosis based on the guideline of traditional herbal medicine. Most of the symptoms were remarkably improved after 60 days of the herbal treatment. One year after discontinuation of the treatment, she complained of gait ataxia. She was treated with the modified Chinkan-sokufu-to for 60 days. Gait ataxia was markedly improved by the second treatment. 15 months after discontinuation of the second treatment, she complained of gait ataxia again. The same remedy was given for 60 days. Gait ataxia was remarkably reduced again. Her total ataxia score was improved from 20 to 3 on a 100-point semiquantitative International Cooperative Ataxia Rating Scales(ICARS). Another case was a 75-year-old Japanese male suffered from unsteadiness of gait, ataxia of gait and stance and positional vertigo. Head MRI revealed a typical atrophic image in cerebellum. Genetic tests revealed an expanded allele of 22 CAG repeats at the spinocerebellar ataxia type 6 locus. He was diagnosed as SCA 6. A mixture of 18 medicinal herbs (modified Chinkan-sokufu-to) was given. Two weeks after the administration of the medicinal herbs, positional ataxia disappeared. Most of the symptoms were remarkably improved after 60 days of the herbal treatment. Together with the first case, the results imply therapeutic potential of the medicinal herbs for spinocerebellar ataxia 6.

INCREASED DNA DAMAGE SENSITIVITY IN SMC1A-MUTATED CORNELIA DE LANGE SYNDROME CELLS. *A. Musio[1, 4], M. L. Focarelli[2, 3], M. Paulis[2, 3], P. Vezzoni[2, 3]* 1) Istituto di Tecnologie Biomediche, CNR, Pisa, Italy; 2) Istituto di Tecnologie Biomediche, CNR, Segrate (Mi), Italy; 3) Istituto Clinico Humanitas, Rozzano (Mi), Italy; 4) Istituto Toscano Tumori, Florence, Italy.

Sister chromatids cohesion is a fundamental aspect of chromosome behavior that ensures the accurate inheritance of the genetic information during mitosis and meiosis. The central player of this process is cohesin, a protein complex composed of a heterodimer of SMC1A and SMC3, associated with SCC1 and SCC3/SA3. Several other proteins (NIPBL, ESCO2, PDS5A, PDS5B) are involved in the loading, unloading, maintenance and function of this complex. The finding that cohesin participates in a growing assortment of chromosome-related processes provides the possibility that new roles for cohesin factors will be discovered. Therefore it would not be surprising if cohesin complex members are involved in human diseases. Recently, we found that mutations in SMC1A are responsible for a subset of Cornelia de Lange syndrome (CdLS), accounting for 5% of cases. CdLS is a multisystem developmental disorder with classic features of characteristic facial dysmorphia, upper extremity malformations, hirsutism, cardiac defects, growth and cognitive retardation, and gastrointestinal abnormalities that display a wide spectrum of clinical severity. Furthermore, some CdLS patients developed metaplasia esophagus, Barrett esophagus and esophageal adenocarcinoma, suggesting a genetic predisposition to esophageal alterations in CdLS. Results from our laboratory showed that SMC1A-mutated patients have spontaneous genome instability and cells are sensitive to genotoxic agents further supporting the notion that SMC1A play a role in genome maintenance.

Polymorphisms of genes in dopamine pathway determine susceptibility to Parkinsons disease and may influence mortality and aging. *Y. Fang[1], Z. Bochdanovits[1], P. Rizzu[1], D. Sondervan[1], D. Deeg[2], N. van Schoor[2], B. Post[3], J. van Hilten[4], P. Heutink[1]* 1) Dept Clinical Genetics, Free University Medical Center (VUMC), Amsterdam, Netherlands; 2) EMGO institute, VUMC, Amsterdam, Netherlands; 3) Dept of Neurology, Academic Medical Center, Amsterdam, Netherlands; 4) Dept of Neurology, LUMC, Leiden, Netherlands.

Parkinsons disease (PD) is a complex genetic disorder with an unclear etiology. Parkinsonism appears when dopamine in the neural cells decreases. Dopamine and its metabolic pathway play a crucial role in the PD pathological progress. We systematically investigated the genetic effect and interaction of sequence variation in genes of the dopaminergic pathway for the risk of PD. With our selection criteria we selected 145 out of 1918 single nucleotide polymorphisms (SNPs) across six major genes in the pathway. 127 SNPs with calling rate95%; and in Hardy-Weinberg equilibrium were successful genotyped in two independent Dutch PD cohorts and a control cohort. We identified 3 SNPs: rs10743152 in the promoter region of the TH gene; non-synonymous SNP, rs4531, in the DBH gene and rs11575575 from 3-end of the DDC gene, to be significantly associated with increased risk of PD in combined late-onset PD cohort (n=313) versus age and gender-matched control cohort (n=706).The T-allele carriers of those SNPs increased 1.52, 1.34 and 1.65 times risk of PD respectively (all p0.001). The same PD-risk genotype of the rs10743152 was associated with 2.6 years decreased age (p0.001) and increased 30% risk of morality (p=0.005) compared to non T-allele carriers in the control cohort (n=1319). We observed an interaction between rs10743152 of TH gene and rs4531 of the DBH gene for the risk of PD (p=0.04). No interaction (p=0.52) but an additive effect between the T-carrier of rs10743152 and the non 266-carrier of the SNCA-REP1 repeat of the SNCA gene for the risk of PD was found: two risk genotype carriers increase 2.8 times risk of PD compared to non risk genotype carriers (p0.001). Our results indicate that TH might affect the normal aging process, and the risk of develop PD is a consequence of the age related neurological deterioration associated with TH.

Study of audiometric measurements using principal components analysis. *E. Fransen[1], J. R. Huyghe[1], O. Thas[2], L. Van Laer[1], G. Van Camp[1]* 1) Center for Medical Genetics, University of Antwerp (CDE), Antwerp, Belgium; 2) Departement of Applied Mathematics, Biometrics and Process Control, University of Gent (UGent), Ghent, Belgium.

The most important technique to test an individuals hearing capability is a pure-tone audiometry (PTA). A test subject has to respond to sound stimuli of a certain sound level (in decibel, db) and a well-defined frequency (conventional including 0.25, 0.5, 1, 2, 4 and 8 kiloHerz (kHz). At each frequency, one records the lowest sound level that the patient responds to, which is referred to as the hearing threshold. Our research interest is the elucidation of genetic and environmental risk factors for age-related hearing impairment. Typically, a statistical association is tested between the hearing phenotype and the putative risk factor. On the phenotypic side, we have to deal with two complicating factors: First, age and sex are two covariates we have to account for. Second, we want to reduce the dimension of our thresholds recording (at six frequencies) to a lower-dimensional observation. To correct for age and sex, linear regression models were fitted at each of the different frequencies, regressing log-transformed hearing threshold on age + age². Then, principal components (PC) analysis was used to reduce the dimension of this six-variate observation. The first PC was a size component accounting for 55% of the variance. The second PC showed a contrast between subjects with a uniform hearing loss across all frequencies (flat audiogram) and subjects with hearing loss in high frequencies only (sloping audiogram). The third PC identified a subgroup of subjects with hearing loss restricted to 2 and 4 kHz, an audiometric pattern that is the hallmark of noise-induced hearing loss. These different types of hearing loss are possibly correlated to different pathological changes in the inner ear. Using the PCs, we have reanalyzed the role of environmental risk factors and candidate genes on age-related hearing loss.

Large BRCA1 and BRCA2 genomic rearrangements in Belgian breast-ovarian cancer families. *K. Storm, S. Willocx, W. Wuyts, N. Van der Aa, J. van den Ende, B. Blaumeiser* Dept Medical Genetics, University and University Hospital of Antwerp, Antwerp, Belgium.

Background: BRCA1 and BRCA2 germ-line mutations predispose to breast and ovarian cancer. Most of the alterations are point mutations or small insertions/deletions in BRCA1 or BRCA2. Large genomic rearrangements of BRCA1 account for 0-36 % of all disease causing mutations in various populations, while large genomic rearrangements in BRCA2 are more rare. In our screening approach for the BRCA genes, screening of the complete coding sequence of both genes is completed by MLPA screening for larger genomic rearrangements. While little data can be found on BRCA1 genomic rearrangements in the Belgian population, knowledge of BRCA2 genomic rearrangements in our population is still unknown. Methods: Multiplex ligation-dependent probe amplification (MLPA) was used to analyze 305 Belgian breast and/or ovarian cancer patients (including high-risk families, single early-onset cases, and familial cases with a more modest risk for BRCA mutations) in whom a deleterious mutation in BRCA1 and BRCA2 was not detected by DGGE and/or sequencing analysis. Results: Two different genomic rearrangements were detected in 8 patients: A recurrent exon 13 deletion in BRCA1 was identified in 6 high risk families (~2 %), accounting for 17,1 % (6/35 index cases) of all BRCA1 mutations in our Belgian population. In BRCA2, a novel in-frame exon 19 deletion (within a DNA-binding domain of BRCA2) was characterized in 2 high risk families (~0.7 %), accounting for 7,7 % (2/26 index cases) of our BRCA2 families. Conclusion: In Belgium, women eligible for BRCA1 and BRCA2 mutation screening, when found negative, could benefit from screening for large genomic rearrangements in both genes BRCA1 and BRCA2. Genomic rearrangements should be screened in high risk families, especially when breast cancer occurs together with ovarian cancer.

Assessment of morphological features in BRCA1 and BRCA2 carriers. *S. Shanley[1], K. Bishop[1,2], V. Murday[3], M. Wilmot[3], DGR. Evans[4], D. Eccles[5], S. Hodgson[6], S. Ashley[1], L. Ashcroft[7], A. Tutt[8], E. Bancroft[1], G. Gui and the Breast Unit[1], A. Baildam[7], A. Howell[7], G. Royle[9], D. Easton[10], R. Eeles[1, 11]* 1) Cancer Genetics Unit, Royal Marsden NHS Foundation Trust, Sutton UK; 2) Department of Genetic Medicine, VanderBilt University, Nashville, Tennessee, USA; 3) West Scotland Regional Genetics Service Ferguson Smith Centre for Clinical Genetics Yorkhill NHS Trust Glasgow UK; 4) Academic Unit of Medical Genetics and Regional Genetics Services St Marys Hospital, Manchester UK; 5) Wessex Clinical Genetics Service Princess Anne Hospital Southampton UK; 6) South West Thames Regional Genetics Service St Georges Hospital Cranmer Tce, London UK; 7) Christie Hospital, Withington, Manchester UK; 8) Oncology Unit Guys and St Thomas' NHS Foundation Trust London UK; 9) Department of Surgery, Royal South Hants Hospital, Southampton UK; 10) Strangeways Research Laboratory, Worts Causeway, Cambridge UK; 11) Institute of Cancer Research Downs Rd Sutton UK.

Some cancer predisposition syndromes are associated with specific morphological features which can assist in making a clinical diagnosis eg macrocephaly and mucocutaneous lesions in Cowden Syndrome. No specific features have been noted in association with the BRCA1 and BRCA2 phenotypes but few data have been collected. In a study comparing side effects of breast radiation and chemotherapy in female BRCA carriers versus women with sporadic breast cancer, participants were also invited to have clinical photographs and basic clinical measurements. Clinical photographs were examined independently by two clinical geneticists who were unaware of the mutation status of individuals. Data are presented on 41 pairs of BRCA carriers with control participants who were matched for age and treatment regimens.

Identification of Novel Loci for Insulin Sensitivity and Disposition Index in GWAs of Hispanic Americans: The IRAS Family Study. *N. Palmer[1], C. Langefeld[2], J. Ziegler[2], F. Hsu[2], S. Haffner[3], T. Fingerlin[4], J. Norris[4], Y. Chen[5], S. Rich[6], T. Haritunians[5], K. Taylor[5], R. Bergman[7], J. Rotter[5], D. Bowden[1]* 1) Biochemistry, Wake Forest Univ, Winston-Salem, NC; 2) Public Health Sciences; Wake Forest Univ, Winston-Salem, NC; 3) Medicine; Univ of Texas, San Antonio, TX; 4) Preventive Medicine & Biometrics; Univ of Colorado, Denver, CO; 5) Medical Genetics Institute; Cedar-Sinai Research Institute, Los Angeles, CA; 6) Public Health Genomics; Univ of Virginia, Charlottesville, VA; 7) Physiology & Biophysics; Univ of Sourthern California, Los Angeles, CA.

To date, Genome Wide Association studies (GWAs) of T2D have identified genes for -cell function. We measured insulin sensitivity (S_I), insulin secretion (acute insulin response; AIR) and Disposition Index (DI; quantitative measure of the compensatory relationship between SI and AIR) DI=S_IxAIR) using the frequently sampled intravenous glucose tolerance test with minimal model analysis in non-diabetic Hispanic Americans from the Insulin Resistance Atherosclerosis Family Study. A GWAs (*n*=229, 34 families) was performed with 318K SNPs. Association analysis adjusted for age, gender, center & BMI used a variance component measured genotype approach in SOLAR. SNPs associated with glucose homeostasis measures (*n*=672 SNPs) were genotyped in the entire cohort (*n*=1190, 92 families). Among the top hits for S_I in the combined analysis, VIPR1 (involved in smooth muscle relaxation; P=2.1x10^{-4}), MAGI1 (involved in cell-cell contact; P=3.8 x10^{-4}), 2 SNPs (P<2.2x10^{-4}) upstream of AK097474 (hypothetical gene in muscle & liver) and 3 SNPs in a nongenic region (15q24.1; P<1.8x10^{-3}) were associated. Among the top hits for DI in the combined analysis, SLC1A4 (sodium-dependent solute carrier; P=3.3x10^{-4}) and PGM1 (involved in glucose synthesis & breakdown; P=3.4x10^{-4}) were associated. Noteworthy, MAGI1 and MYH13 (myosin skeletal muscle polypeptide) were in the top 10 genic hits for S_I and DI. In summary, candidate loci for S_I and/or DI in Hispanic Americans have been identified using GWAs. These loci have not been revealed in T2D GWAs in European-derived populations.

Mutation spectrum of the dystrophin gene in 433 Japanese dystrophinopathy cases. *Y. Takeshima, M. Yagi, Y. Okizuka, H. Awano, Z. Zhang, K. Saiki, M. Matsuo* Dept Pediatrics, Kobe University Graduate School of Medicine, Kobe, Japan.

[Background] Duchenne and Becker muscular dystrophies are caused by mutations in the dystrophin gene. Mutation analysis of the dystrophin gene is indispensable not only to provide proper clinical information but to apply the molecular therapies, which depend on the type of mutation in each case. However, the large size of dystrophin gene (3000kb and 79 exons) hampers the detection of small mutations including nonsense, splice site, and small deletion/insertion mutations. To clarify the responsible mutations of all cases, comprehensive mutation analysis of the dystrophin gene employing not only genomic DNA but mRNA was performed in dystrophinopathy cases. [Patients and Methods] A total of 433 Japanese dystrophinopathy cases were recruited. Gross gene arrangements were detected by Southern blotting, MLPA analysis, or semi-quantitative PCR analysis by capillary electrophoresis, and small mutations were analyzed by RT-PCR or direct sequence method using genomic DNA or cDNA as template. [Results] 265 (61%) and 39 (9%) cases had large deletions and duplications encompassing at least one exon, respectively. Small deletion/insertion mutations less than several hundred nucleotides were disclosed in 32 (7%) cases. Nonsense and splice site mutations were identified in 65 (15%) and 28 (6%) cases, respectively. X-chromosome abnormalities were disclosed in 3 cases. In remaining one case, however, no mutation was detected. There were several noteworthy cases as follow. First, 2 cases with duplication mutations had two noncontiguous duplicated regions. Next, 2 cases with insertion sequences composing of ~608bp and ~325bp, respectively had insertions of retrotransposons. In 3 cases deep intronic mutations activating the cryptic splice sites were disclosed. [Conclusions] Comprehensive mutation analysis revealed the responsible gene mutations in 99.8% of the dystrophinopathy cases, and the present result disclosed the highest mutation detection rate.

Gene expression levels in African Americans vary with both cis and trans genetic ancestry. *A. L. Price[1], N. Patterson[2], D. C. Hancks[3], S. Myers[4], D. Reich[5], V. G. Cheung[3,6,7], R. S. Spielman[3]* 1) Harvard School of Public Health, Boston, MA; 2) Broad Institute, Cambridge, MA; 3) University of Pennsylvania, Philadelphia, PA; 4) Oxford University, Oxford, UK; 5) Harvard Medical School, Boston, MA; 6) Children's Hospital of Philadelphia, Philadelphia, PA; 7) Howard Hughes Medical Institute, Philadelphia, PA.

Variation in gene expression is a fundamental aspect of human phenotypic variation. Several recent studies have analyzed gene expression levels in cell lines from populations of different continental ancestry and reported population differences at a large number of genes. However, it is currently unknown to what extent these population differences are due to genetic, environmental, or other effects. To address this, we have analyzed gene expression levels in African-American cell lines, which differ from previously analyzed cell lines in that individuals from this population have variable proportions of continental ancestry. By relating gene expression levels in 89 African Americans to their genome-wide proportion of European ancestry, we validated observed differences between 60 Europeans and 60 Africans, providing strong evidence of genetic effects. Furthermore, we accurately inferred local ancestry (0, 1 or 2 European chromosomes) at each location in the genome using dense genotype data, in order to distinguish the effects of local ancestry at the cis locus of each gene from the effects of trans ancestry at other loci. Both cis and trans ancestry effects were strongly statistically significant. By comparing the magnitude of these effects, we estimate that 12 3% of all genetically heritable variation in human gene expression is due to cis variants.

5,10 Methylenetetrahydrofolate Reductase C677T Gene Mutation Associated Unsuccessful Assisted Reproduction. *B. OZTURK[1], B. YAZAR[2], O. ILBAY[2], H. COMERT[2], N. ERCELEN[2]* 1) Department of Clinical Genetics, East Carolina University Brody School of Medicine, Greenville, NC, USA; 2) Genetics and Genomic Sciences Center, VKV American Hospital, Istanbul, TURKEY.

Thrombophilia has been associated with adverse pregnancy outcomes and recurrent pregnancy loss. However, it may also contribute to assisted reproductive failure (ARF). To determine the association of specific inherited thrombophilias and ARF, the prevalence of factor V Leiden (FVL), prothrombin G20210A and 5,10 Methylenetetrahydro-folate Reductase (MTHFR) C677T gene mutations were investigated. A consecutive series of 14 women with ARF was enrolled in the study group. Control group was 40 women with at least one successful pregnancy and no history of pregnancy loss. Mean age of the study group was 33.3 years (range, 28-41) versus 33.8 (range, 24-45) in control group. Mean number of ARF was 1.9 (range,1-5) in the study group. At least one thrombophilic defect was found in 85.7 % of total study group women compared with 57.5 % in controls (p<000.1, OR: 4.448, 95 % CI: 2.2-8.8). Presence of MTHFR mutations was associated with an extremely significant increased risk for ARF (71 versus 43%, p= 0.0001, OR: 3.245, 95 % CI: 1.8-5.8). The presence of FVL mutation showed no significant increased risk for ARF (14 versus 13%, p= 1.0000, OR: 1.167, 95 % CI: 0.1-6.8). In addition none of the patients in the study group had prothrombin mutation (p= 1.0000, OR: 0.9080, 95 % CI: 0.03-23.6). C677T MTHFR mutation might be a risk factor for ARF especially in younger age women. Definitive conclusions require analysis of larger series to confirm the result.

Genetic Similarity Matching for Genome-wide Association Studies with Related Individuals. *W. Guan, L. Liang, M. Boehnke, G. R. Abecasis* Dept Biostatistics, Univ Michigan, Ann Arbor, MI.

Recently, genome-wide association (GWA) studies have drawn great interest as a promising tool to dissect the genetic basis of complex diseases such as hypertension, diabetes, and bipolar disorder. Population stratification is a major concern that can lead to spurious disease-marker association or mask a true association. We have proposed a similarity score matching method that matches cases and controls based on their identity-by-state similarity scores using the large amount of genotype data from GWA studies. We now extend our method to the analysis of related case and control samples. We apply a new test statistic, based on the work of Thornton et al. 2007, to correct the correlation between the related samples and matched case-control pairs. Through computer simulations, we demonstrate that our method correctly controls type I errors, and has improved power compared to genomic control in the presence of stratification. We illustrate our method with data from the Pritzker Consortium Bipolar GWA study.

Homozygous R316Q mutation in the obesity-associated FTO gene causes a severe polymalformative syndrome. *S. Boissel[1,3], O. Reish[2,3], F. Molinari[1], N. Kadhom[1], C. Golzio[1], H. Etchevers[1], A. Munnich[1], L. Colleaux[1]* 1) INSERM U781, Université Paris Descartes, Paris, France; 2) Department of Medical Genetics, Assaf Harofeh, Israel; 3) Equally contributors.

We report on a large inbred family including 9 affected children presenting a novel syndrome characterized by severe intra-uterine growth and psychomotor retardation, delayed myelinization, cleft palate, cardiac and genital anomalies, hypertonicity and premature death. Extensive metabolic and genetic workup were normal. As the pedigree suggested an autosomal recessive mode of inheritance, autozygosity mapping was performed identifying a unique 6.5 Mb long region of shared homozygosity on 16q12 between D16S411 and D16S3140 markers. We identified a homozygous missense variation within the FTO gene (c.947G>A, p.R316Q), which co-segregated with the disease, was not found in 400 control alleles and altered a highly conserved residue. While recent studies have revealed a strong association between intronic variants in FTO and obesity, the pathophysiological mechanism underlying the phenotype observed in our family remains questionable. FTO encodes a 2-oxoglutarate-dependent nucleic acid demethylase involved in DNA and/or RNA lesions repair. The R316 residue is involved in iron- and 2OG-coordination and a recent study showed that a R316A substitution abolishes Fto activity in-vitro. To further understand the consequences of the p.R316Q variant, western blotting and immunofluorescence experiments were performed on patient and control fibroblasts. We found that the amount of FTO protein is significantly reduced in patient cells even though the nuclear localization is not affected. Moreover, patient fibroblasts display signs of senescence including growth defects, limited replicative lifespan and altered cell morphology. Mass spectrometry assays and cellular sensitivity to methylating agents measurements are underway to further characterize this cellular defect. To conclude, our data suggest that homozygous FTO null mutations are responsible for a newly described polymalformative syndrome. They also provide the first example of a disorder related to a recently identified nucleic acid repair pathway.

Validation of the association between the *COL1A1* polymorphism and high myopia in the Japanese population.

H. Nakanishi[1,2], R. Yamada[2,3], N. Gotoh[1,2], H. Hayashi[1,2], A. Otani[1], A. Tsujikawa[1], K. Yamashiro[1], N. Shimada[4], K. Ohno-Matsui[4], M. Mochizuki[4], M. Saito[5], K. Saito[5], T. Iida[5], F. Matsuda[2], N. Yoshimura[1] 1) Ophthalmology and Visual Sciences, Kyoto University Graduate School of Medicine, Kyoto, Japan; 2) Center for Genomic Medicine, Kyoto University Graduate School of Medicine, Kyoto, Japan; 3) Human Genome Center, Institute of Medical Science, University of Tokyo, Tokyo, Japan; 4) Ophthalmology and Visual Science, Tokyo Medical and Dental University Graduate School, Tokyo, Japan; 5) Ophthalmology, Fukushima Medical University, Fukushima, Japan.

The collagen type alpha 1 (*COL1A1*) gene encodes the pro-alpha1 chains of type collagen and is located on chromosome 17q21.33, where a myopia susceptibility loci (MYP5, 17q21-22) has been previously reported. Type collagen is a major component of sclera, remodeling of which is a characteristic finding of a highly-myopic eyeball wall. The *COL1A1* was recently reported to be associated with high myopia in the Japanese population. To validate this positive association, we conducted a systematic case-control association study using the tagging single nucleotide polymorphism (tSNP) approach. Eight tSNPs, including rs2075555 and rs2269336 (previously reported to be high myopia-susceptible SNPs in Japanese), were selected to tag the linkage disequilibrium blocks harboring the *COL1A1*. These tSNPs were genotyped using the Taqman SNP assay. A total of 427 unrelated Japanese cases with high myopia (axial length 26.50 mm in both eyes) and 420 Japanese controls were recruited. There was no association noted between high myopia and rs2075555 (P=0.47, Pc0.99) and rs2269336 (P=0.40, Pc0.99). No significant associations were seen with further tSNPs tests. This study did not replicate the previously reported positive association between the *COL1A1* and high myopia in the Japanese population. To elucidate whether the *COL1A1* gene in the MYP5 locus is associated with high myopia in the Japanese population, additional genetic and molecular biological studies are needed.

IVF is a risk factor for small intestinal stenosis/atresia; A population-based study of Kanagawa Birth Defects Monitoring Program in Japan, 2001-2005. *K. Kurosawa[1], Y. Kuroki[1], T. Yasojima[2]* 1) Kanagawa Children's Med Ctr, Yokohama, Japan; 2) Kanagawa Association of Obstetricians and Gynecologists, Kanagawa, Japan.

Intestinal stenosis/atresia is one of the most common birth defects, characterized by the complete or partial occlusion of the lumen of a segment of intestine with a variety of clinical signs including abdominal distention, bilious vomiting, and polyhydroamnios. Underlying causes for the atresia/stenosis, especially in the region of small gut, include malrotation, meconium ileus, cardiovascular anomalies, and Down syndrome. The etiological mechanism represents vascular disruptions. Data from the Kanagawa Birth Defects Monitoring Program (KAMP) were used to identify infants with small intestinal stenosis/atresia. Case records with a code for intestinal stenosis/atresia were reviewed to substantiate the diagnosis of small intestinal stenosis/atresia and analyze the associated malformations in the cases. As the information on pregnancy by the assisted reproductive techniques of the cases were recorded only in the cases with multiple births, analysis and comparison were made in the case groups according to the singleton, multiple births with IVF, and multiple births with non-IVF. We identified 30 infants born with small intestinal stenosis/atresia in Kanagawa Prefecture during 2001-2005. The birth prevalence was 2.12 per 10,000 births, results consistent with findings from other population-based studies using case definitions. Of 30 cases, we identified 4 cases born after IVF. In the group of multiple births including 1710 cases, all the 2 cases with the disorder were born after IVF. Even though the prevalence of malformed infants born in multiple births significantly higher than those born from singleton, we could not identify the cases with the disorder from 1119 cases born after spontaneous non-IVF. These results suggest that the IVF is a risk factor for small gut stenosis/atresia. Even though the risk for small gut stenosis/atresa after IVF has only a small practical consequence, further analysis is important for considering the etiological mechanisms in the disorder.

Genetic Regulation of Cervical Cytokine Concentrations by Toll-like Receptors. *K. K. Ryckman[1,2], S. M. Williams[1,2], M. A. Krohn[3], H. N. Simhan[3]* 1) Center for Human Genetics Research, Vanderbilt University, Nashville, TN; 2) Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN; 3) Magee-Womens Research Institute, University of Pittsburgh School of Medicine, Pittsburgh, PA.

Objective: Toll-like receptors (TLRs) are critical components of the innate immune system and function not only in recognizing bacterial micro-organisms but also in inducing local expression of pro- and anti- inflammatory cytokines. Therefore, the purpose of this study was to assess the impact of genetic variation in TLR genes on cervical concentrations of pro- and anti- inflammatory cytokines and to determine if this relationship is influenced by bacterial vaginosis (BV). Study Design: Four single nucleotide polymorphisms (SNPs) in TLR2 and thirteen in TLR4 were examined for associations with eleven cervical pro- and anti- inflammatory cytokine levels in 52 black and 64 white women. Results: White, but not black, BV negative women with the CC/CT genotype at rs1554973 have lower cervical levels of interleukin-1 beta (IL-1) compared to women with the TT genotype. Additionally, in white women rs1927911 and rs2149356 are associated with cervical IL-1, interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-) concentrations. Importantly, these associations are affected by BV status. For example, women with the CC genotype at rs1927911 or rs2149356 have higher cervical levels of IL-1 and IL-6 than women with the CT/TT or AA/AC genotypes, respectively; however, this association is only present in BV negative women. In contrast individuals with the CC genotype at rs1927911 or rs2149356 have higher cervical concentrations of TNF- than women with the CT/TT or AA/AC genotypes, respectively; an association that is only present in BV positive women. Conclusion: Our study demonstrates that polymorphisms in the TLR4 gene are associated with pro-inflammatory cervical immune responses, particularly in the context of bacterial vaginosis. Additionally, these patterns of association are different in black and white women.

Association of the HLA-A, -B, -C alleles with severity of malaria in Thailand. *K. Hirayasu[1,2,3], J. Ohashi[1], K. Kashiwase[3], T. Ichihara[3], M. Minemoto[3], H. Hananantachai[4], A. Ogawa[3], M. Takanashi[3], K. Tokunaga[1], J. Patarapotikul[4], T. Yabe[3]* 1) Department of Human Genetics, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan; 2) JSPS Research Fellow; 3) Tokyo Metropolitan Red Cross Blood Center, Tokyo, Japan; 4) Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.

[Purpose] Malaria is one of the most important infectious diseases, affecting 300 million people and causing more than 1 million deaths annually worldwide. Although HLA-B53 was reported to be associated with resistance to severe malaria in West Africans, comprehensive analysis of HLA class I polymorphism in the pathogenesis of malaria severity has not been fully described yet. In this study, we examined a possible association of HLA-A, B, and C alleles with severity of Plasmodium falciparum malaria.

[Methods] 203 mild malaria, 165 non-cerebral severe malaria, and 109 cerebral malaria patients living in northwest Thailand near the border with Myanmar were enrolled in this study. High-resolution HLA typing was performed by Luminex Multi-Analyte Profiling system with a WAKFlow HLA typing kit. Samples showing ambiguous patterns were subjected to sequencing-based typing. The carrier frequencies of the HLA alleles were compared between pairs of malaria patient groups using the Fishers exact test based on a 2 x 2 contingency table.

[Results] In our study population, a total of 34, 62, and 23 alleles were detected at HLA-A, B, and C loci, respectively. Furthermore, a novel HLA-B allele has been identified. In addition, a weak association was observed between HLA-B*4601 and cerebral malaria (P=0.01), consistent with previous studies of the Myanmar population (Tokai J Exp Clin Med 23(2):81-83, 1998), and our low-resolution HLA data in Thailand (Jpn J Infect Dis 58:25-28, 2005). Further analyses are currently under investigation.

Genomewide linkage and fine mapping identifies ELP4 as a major susceptibility gene for Rolandic epilepsy in two independent samples. *D. K. Pal[1], L. J. Strug[2], T. Clarke[1], B. Bali[1], T. Chiang[4], M. Chien[2], J. J. Russo[3]* 1) Dept Psychiatry, Columbia University, New York, NY; 2) Child Health Evaluative Sciences, The Hospital for Sick Children, Toronto, Canada; 3) Columbia Genome Center and Dept of Chemical Engineering, Columbia University, New York, NY; 4) Centre for Computational Biology, The Hospital for Sick Children, Toronto, Canada.

Introduction. Rolandic epilepsy (RE) is the commonest human epilepsy accounting for 15-20% of all new cases. It is defined by the EEG trait of centrotemporal sharp waves (CTS). A genomewide linkage screen for CTS in 38 families, ascertained through an RE proband, identified a major CTS locus at 11p13 (multipoint lod 4.30) at marker D11S914. We fine mapped this linkage region. **Methods**. We collected DNA from 50 RE cases and 190 controls free of neuropsychiatric disorders, matched for sex and ethnicity. 32 genic tag SNPs were typed around the linkage peak and a further 12 SNPs typed in and around the region of maximal association. A subset of 29 SNPs was typed in an independently collected Canadian sample of 40 RE cases and 137 controls. Genotyping was performed blind to center and caseness by deCODE Genetics (Iceland). **Results**. The US dataset revealed strong evidence of association with four SNPs in the Elongator complex protein 4 (*ELP4*) gene. rs964112 in intron 3-4 provided the smallest p-value (0.00084), significant after Bonferroni correction (OR=2.13, 95% CI 1.35-3.45). In the replication sample, the same gene was implicated, the smallest p value occuring 105 kB away at rs2104246 in *ELP4* intron 9-10 (OR=2.92 95%CI 1.55-5.52, p=0.00062). Resequencing 40 RE cases, we found no coding region mutations. **Discussion**. We present evidence from two independent datasets that variants in *ELP4* are associated with the CTS trait, strongly predisposing to Rolandic epilepsy. *ELP4* is a component of Elongator, which associates with RNA polymerase II. *ELP4* is highly expressed during fetal development in forebrain areas implicated in the developmental pathology of RE including frontal cortex, basal ganglia and hippocampus. The lack of a coding sequence mutation suggests a possible fault in regulatory control.

Prenatal diagnosis of trisomy 18 mosaicism: 24 new cases and review of the literature. *L. Ballarati[1], L. Camurri[2], G. Nocera[3], L. De Grada[1], C. Corti[1], L. Larizza[1,4], D. Giardino[1]* 1) Lab. Citogenetica Medica e Genetica Molecolare IRCCS Istituto Auxologico Italiano Milano, Italy; 2) Arcella Analisi Mediche GENIMED Padova, Italy; 3) SS di Citogenetica Clinica Ostetrico Ginecologica Università di Milano AO Fatebenefratelli e Oftalmico Milano, Italy; 4) Genetica Medica Dip. Di Medicina Chirurgia e Odontoiatria Universita di Milano, Italy.

Dysomy-trysomy mosaicism could derive from a mitotic postzigotic nondisjunction event in an originally euploid zygote or from a postzigotic loss of a supernumerary chromosome in an aneuploid zygote. Disomy-trisomy chromosomal mosaicism prenatally detected on Chorionic Villus Sampling (CVS) could be represented by: I) the simultaneous presence of a disomic and a trisomic cell line in either cytotrophoblast, mesenchyme or both, or II) the presence of a non mosaic trisomy in the cytotrophoblast concomitant with a homogeneous disomic cell line in the mesenchyme or viceversa. The finding of mosaicisms on CVS raises problems for diagnosis and counselling, because they may not reflect the fetal chromosomal constitution. We report on a total of 24 cases recruited from 3 Italian laboratories found to bear trisomy 18 mosaicism on CVS. 21 cases belong to group I, while only 2 to group II. In the remaining case only direct preparation could be performed. As regards group I, the direct preparations showed a trisomic mosaic in 18 out of 21, with a long-term culture karyotype homogeneously euploid in 13, homogeneously aneuploid in 2 and mosaic trisomic in 3 cases. In the remaining 3 cases, the direct preparation karyotype was homogeneously euploid, whereas the culture showed a trisomy 18 mosaic karyotype. In the 2 cases belonging to group II the cytotrophoblast was euploid and the mesenchyme showed a homogeneous 18 trisomy complement. After genetic counselling, all cases were addressed to further investigation on amniotic fluid samples. Our findings indicate a confined placental mosaicism (CPM) in 16 cases and a true fetal mosaicism (TFM) in 5. No information on the pregnancies outcome was available in the remaining 3 cases. Our data will be discussed and compared to the previously reported cases in an attempt to better define the TFM-related risk.

Complex chromosome 18p rearrangements in two patients with immunological disorders. *M. P. Recalcati[1], E. Valtorta[1], L. Romitti[2], M. Manfredini[3], R. Vaccari[4], L. Larizza[1,5], P. Finelli[1,6]* 1) Laboratorio di Citogenetica Medica e Genetica Molecolare, Istituto Auxologico Italiano, Milano; 2) S.C. Anatomia, Istologia patologica Citogenetica, Ospedale Niguarda Ca Granda, Milano; 3) S.C. Ostetricia e ginecologia, Ospedale Niguarda Ca Granda, Milano; 4) S.C. Neuropsichiatria Infantile, Ospedale Niguarda Ca Granda, Milano; 5) Laboratorio di Genetica Medica, Ospedale San Paolo, Università of Milan, Milano; 6) Dipartimento di Biologia e Genetica per le Scienze Mediche, Università di Milano, Milano.

We report on the cases of two female patients bearing complex rearrangements involving chromosome 18p. Molecular cytogenetic analyses, performed by array-CGH and BAC FISH enabled the precise identification of the affected18p region with the novel order of the genomic sequences . The first patient has a 2 Mb terminal deletion associated with an 8,7 Mb inverted duplication of the adjacent region, while the latter has a more extended 10 Mb terminal deletion associated with a 4,5 Mb quadruplication of the adjacent region and a 1,7 Mb duplication of the pericentromeric region. The patients share dysmorphic features typical of the 18p- syndrome, such as growth retardation, epicanthal folds, long philtrum and toes defects, but suffer from opposite immunological disorders. The first patient has a form of immunological deficiency that manifests with recurrent pulmonary infections and low level of IgA, while the other suffers from an autoimmune form of juvenile rheumatoid arthritis (JRA). Deletion of chromosome 18pter has been linked to immunological disorders and a number of , patients have been described showing the tendency to develop autoimmune disorders including juvenile diabetes, rheumatoid arthritis, thyroiditis, Graves disease; however lack of reduction of serum IgA has been also frequently reported. The relationships between 18p terminal deletion and immunity have been so far not fully explored (dissected/deepened). The refined molecular cytogenetics characterization of different 18p chromosomal rearrangements versus patients specific clinical evaluations could foster our understanding on the role of the 18p region in the immune response.

A new autosomal recessive syndrome with death in early childhood: Wide anterior fontanel, sparse hair, prominent forehead, thickened eyelids, hypoplastic maxilla and late-erupting teeth. *E. Utine, Y. Alanay, D. AktaÅŸ, K. BoduroÄŸlu, M. AlikaÅŸifoÄŸlu, E. Tunçbilek* Clinical Genetics, Pediatrics, Hacettepe Univ, Ankara, Turkey.

Four patients are presented from three distinct families, with strikingly similar facial features, very sparse and fine hair, delayed closure of anterior fontanel and delayed eruption of teeth. Facial features included long face with frontal prominence, thickened eyelids, hypertelorism and epicanthic folds, hypoplastic maxillae, long and deeply grooved philtrum with a thin upper lip, and prominent and low-set ears. All four had a low-normal birth weight and failure to thrive, with delay in fontanel closure and dentition despite chronologically appropriate bone age. Three of them had mild delay in developmental milestones. Three of the patients died after severe clinical courses, presenting with systemic hypertension and renal dysfunction in two and with pulmonary hypertension in one. Parental consanguinity was present in all three families. To the best of our knowledge, this association had not been previously reported, and striking similarity in facial features and clinical findings of these four patients, born to three distinct families with parental consanguinity, suggests a possibility of a newly recognized syndrome with autosomal recessive inheritance.

ADAMTSL2 mutations in geleophysic dysplasia are responsible for a dysregulation of TGF pathway. *C. Le Goff[1], F. Morice-Picard[1], N. Dagoneau[1], L. W. Wang[2], C. Perrot[1], Y. J. Crow[3], F. Bauer[4], E. Flori[5], C. Prost-Squarcioni[6], D. Krakow[7], G. Ge[8], D. S. Greenspan[8], D. Bonnet[9], M. Le Merrer[1], A. Munnich[1], S. S. Apte[2], V. Cormier-Daire[1]* 1) Dept of Genetics, INSERM U781, Hôpital Necker, Paris, France; 2) Department of Biochemical Engineering, Lerner Research Institute, Cleveland, USA; 3) Leeds Institute of Molecular Medicine, Leeds, UK; 4) Clinique Paofai, Fare Tony, Papeete, Tahiti; 5) Dept of Genetics, Hôpital de Hautepierre, Strasbourg, France; 6) CNRS UPRES 3410, Hôpital Avicennes, France; 7) Medical Genetics Institute, Cedars-Sinai Medical Center, Los Angeles, USA; 8) Department of Pathology and Laboratory Medicine, University of Wisconsin, Madison, USA; 9) Service de Cardiologie Pédiatrique, Hôpital Necker, Paris, France.

Geleophysic dysplasia (GD, OMIM 231050) is an autosomal recessive disorder characterized by short stature, brachydactyly, thick skin, hepatomegaly and cardiac valvular anomalies often responsible for an early death. Studying six GD families, we first mapped the disease gene to chromosome 9q34.2 (Zmax = 4.52 at = 0 at the gt-AL590710 locus) and identified five distinct nonsense/missense mutations in the A Disintegrin And Metalloproteinase with Thrombospondin repeats- like 2 gene (ADAMTSL2), which encodes a secreted glycoprotein of unknown function. In situ hybridization of human foetal tissues showed that ADAMTSL2 is widely expressed, particularly in tissues involved in GD (skin, heart and growth plate). Functional studies in HEK293 cells showed that ADAMTSL2 mutations lead to a decreased secretion of the mutated proteins, possibly due to the misfolding of ADAMTSL2. A yeast two-hybrid screen revealed that ADAMTSL2 interacts with Latent TGF Binding Protein 1 (LTBP1), which plays a major role in the storage of latent TGF- in the extracellular matrix and regulates its availability. To determine the functional significance of this interaction, we quantitated TGF- and found a significant increase of total and active TGF in the culture medium and the nuclear localization of phosphorylated Smad2 in GD fibroblasts. These data suggest that ADAMTSL2 mutations may lead to a dysregulation of TGF signalling as the underlying mechanism of GD.

The del(8)(p23) syndrome and aniridia. *J. G. Pappas[1], K. E. Daley[1], I. K. Gadi[2]* 1) Dept Pediatrics,Human Genetics, New York Univ, Sch Medicine, New York, NY; 2) Laboratory Corp America, Dept. Cytogenetics, Research Triangle Park, NC.

We present a 17-month-old boy with terminal microdeletion 8p23.2. He was born 38.5 weeks of gestation anthropometrically appropriate for gestational age. At 2.5 months, his weight was slightly over the 97th centile, his length and occipitofrontal circumference (OFC) was between the 25th and 50th centile, he had increased tone of all extremities, palpable metopic ridge, up-slanted palpebral fissures, aniridia, nystagmus, low set posteriorly rotated ears with over-folded upper helices, small upturned nose, down-turned corners of the mouth, hypoplasia of the nails on the fifth digits in hands and feet, small umbilical hernia and diastasis recti. There was no family history of aniridia or other ocular abnormalities. Chromosome analysis revealed an apparently normal 46,XY karyotype at 500 GTG band resolution. Comparative genomic hybridization array (aCGH) analysis with 607 locus specific BAC clones revealed decreased fluorescence of five BAC clones specific to segment 8p23.2 to 8pter. Fluorescence in situ hybridization (FISH) with an 8p subtelomeric probe confirmed the deletion in one 8p homologue. Karyotype and FISH with the 8p subtelomeric probe in the parents were normal. At 17 months our patient had microcephaly, mild developmental delays and dysmorphic features. This clinical presentation is common in the del(8)(p23) syndrome (Hutchinson R et al, 1992; Paez MT et al 2008; Bert BA et, 2001). del(8)(p23) that encompasses the GATA4 gene is associated with congenital cardiac malformations (Devriendt K et al, 1999). In our case, the GATA4 gene was not included in the deleted segment according to aCGH and there were no cardiac defects by echocardiography. Aniridia has not been reported in the medical literature as a feature of the del(8)(p23) syndrome. The 11p deletion associated with WAGR was not seen in our case and clinically there were no features of any known syndrome associated with aniridia. Our case further supports the hypothesis that the haploinsufficiency of GATA4 is the cause of cardiac defects in the del(8)(p23) syndrome and suggests that aniridia may be a feature of this syndrome.

Type II chaperonin BBS genes, BBS6 and BBS12, are required for BBSome formation. *Q. Zhang, S. Seo, V. Sheffield* Dept Pediatrics, HHMI, Univ Iowa, Iowa City, IA.

Bardet Biedl Syndrome (BBS) is a pleiotropic disorder with primary feature of obesity, retinal degeneration, polydactyly, kidney abnormalities, and cognitive impairment. Other features include hypertension and diabetes. To date, twelve BBS causing genes have been identified through genetic linkage, homozygosity mapping, and comparative genomic analysis. BBS1, BBS2, BBS7 and BBS9 contain beta propeller domains, BBS4 and BBS8 contain multiple TPR domains, BBS5 has two Boo domains, BBS3 has an arf-like domain, BBS11 is an E3 ubiquitin ligase, and BBS6, BBS10, and BBS12 have type II chaperonin like domains. Knockout mice studies together with studies in other model organisms have demonstrated that BBS genes have cilia related functions. Recently seven of the twelve BBS proteins (BBS1, 2, 4, 5, 7, 8, and 9) have been shown to form a complex called the BBSome. The function of the BBSome involves ciliary membrane biogenesis. However little is known about the function(s) of the three chaperone-like BBS genes. Using tissues from BBS knockout mice generated in our laboratory, we show that Bbs1, Bbs2, and Bbs7 protein levels are greatly decreased in Bbs6 knockout mice. There is little change in mRNA levels in these knockout mice. These data suggest that Bbs6 plays a role in stabilizing Bbs1, Bbs2, and Bbs7 proteins. The presence of a beta propellar domain in Bbs1, Bbs2 and Bbs7 suggests that Bbs6 plays a role in the proper folding of the beta propeller structures shared by these three BBS proteins. Biochemical analysis in cultured cells demonstrate that Bbs6, Bbs12 and Bbs7 are co-immunoprecipitated. Bbs7 is required for Bbs6 and Bbs12 interaction, and Bbs6 is required for Bbs7 and Bbs12 interaction. These data indicate that mutation of the chaperonin-like BBS genes, Bbs6 and Bbs12, disrupt BBSome formation by affecting three BBSome subunits Bbs1, Bbs2, and Bbs7 protein stability.

Bilateral oblique facial clefts and extremity anomaly following first trimester efavirenz and other antiretroviral exposures. *A. Shanske[1], K. Beckerman[2], R. Wright[2], Shanske* 1) Depts. of Peds, and; 2) Ob-Gyn, Albert Einstein Coll. of Med, Bronx, NY.

Oblique facial clefts are very rare congenital deformities.Craniofacial clefts and amniotic bands may be part of the amnion rupture syndrome. The amniotic cavity expands during the 12th week of gestation pressing against the chorion and obliterating the extraembryonic coelom. Rupture results in compression or band defects. Our patient has both a compression defect of the forearm and band defects of the craniofacial region. The infant girl was the 2945 gm product of a 37 week gestation complicated by advanced maternal HIV and gestational diabetes. A bilateral cleft lip and palate and swollen left forearm were noted by ultrasound. She was delivered by NSVD to a 35 year old mother who has been HIV positive for 18 years. Her viral load was undetectable during the pregnancy. She received zidovudine/lamivudine and efavirenz before pregnancy through 9 weeks gestation when she was changed to zidovudine/lamivudine and lopinavir/ritonavir and insulin was added for her diabetes. The newborn examination revealed bilateral oblique facial clefts (a Tessier cleft number 3 on the left and number 4 on the right) and right microphthalmia with an ectopic right lacrimal duct protruding from the right inner canthus. The HC was 33.5 cm. A circumferential constriction band encircled the left forearm. Efavirenz (EFV)is a highly potent non-nucleoside reverse transcriptase inhibitor in wide use. Because of a high number of craniofacial defects in cynomolgus monkeys, it is the only antiretroviral that is rated by the FDA Pregnancy Category D. A recent study of data collected in the Antiretroviral Pregnancy Registry, www.APRegistry.com, found no increase in birth defects in 4,000 prospectively monitored 1st trimester exposures compared with 2nd and 3rd. Prospective monitoring of 364 EFV-exposed pregnancies did not show an increase in the prevalence of birth defects after 1st trimester exposure. This child represents the first association of amniotic rupture sequence after EFV exposure and highlights the importance of prospective evaluation of 1st trimester exposures to this potential teratogen.

Cell populations of Human Anterior Capsular Plaque. *J. Kaid, AR. Vasavada* Iladevi Cataract & IOL Research Centre, Ahmedabad-380052, Gujarat, India.

Anterior capsular plaque (ACP) is a type of cataract located just below the anterior surface of the crystalline lens and develops due to abnormal differentiation of lens epithelial cells (LECs). Little is known about the form of abnormal differentiation of cells and hence the origin of the ACP. In present study the cell populations of congenital human ACP were studied based on the location and differentiation of cells. Differentiation study was performed using antibodies against pax6 for undifferentiated LECs, -smooth muscle actin (SMA) for epithelial mesenchymal transition, -crystallin for fiber cell differentiation along with collagen type I, collagen type IV and -crystallin. The human ACP revealed non-plaque and plaque region. The non-plaque region was largely consisted of pax6 positive cells. Within the plaque region, surface cells were positive to pax6 while the cells suspended in the extracellular matrix were either positive to SMA or -crystallin. Most of the cells of plaque were positive for -crystallin and surrounded by a network of collagen type IV. Human ACP consisted of heterogeneous population of undifferentiated and differentiated population of cells. Among the differentiated population an attempt was seen for both epithelial mesenchymal transition and fiber cell differentiation.

Noninvasive Fetal Gender Determination Assay on 3091 Cases. *Y. Wang, K. Thompson* Clinical Mol Diagnostic Lab, Ctr Med Gen, Houston, TX.

Thirty years of practice have shown that prenatal Diagnosis is a powerful weapon against incidents of some lethal diseases passed through inheritance. However, the possibilities of fetal-maternal hemorrhage, infection, and miscarriage caused by the invasive procedures hamper its usefulness in some high-risk pregnant women. The discovery of cell free fetal DNA in maternal circulation has opened up a new avenue for noninvasive prenatal diagnosis. The diagnoses of fetal gender, rhesus D status, and single-gene disorders have been successfully done by analysis of cell free fetal DNA in maternal plasma. The main challenge that remains an obstacle to these molecular noninvasive prenatal diagnoses being accepted as a large scale routine practice is the fact that cell-free fetal DNA presents only 3-6% of the total DNA in maternal plasma. We report here that we have done fetal gender determination assay on 3091 cases in our laboratory using cell free fetal DNA extraction from maternal plasma with Real-Time PCR. Confirmed through ultrasound so far, the accuracy of the test was 98.51% at the beginning, and after technique improving, the accuracy increased to 99%. It is well known that about 1400 genes have been mapped to the X chromosome, 40% of which are known to be associated with disease phenotypes. Fetal Gender determination certainly merits medical significance. Furthermore, the experience which we have obtained from the noninvasive fetal gender determination screening has built a foundation to develop other noninvasive prenatal diagnosis assays and put them on large scale routine clinical tests.

An Unbiased Estimator of Gene Diversity in Samples Containing Related Individuals. *M. DeGiorgio*[1], *N. Rosenberg*[1,2] 1) Center for Computational Medicine and Biology, University of Michigan, Ann Arbor, MI; 2) Department of Human Genetics and the Life Sciences Institute, University of Michigan, Ann Arbor, MI.

Gene diversity is sometimes estimated from samples that contain inbred or related individuals. If inbred or related individuals are included in a sample, then the standard estimator for gene diversity produces a downward bias, caused by an inflation of the variance of estimated allele frequencies. We develop an unbiased estimator for gene diversity that relies on kinship coefficients for pairs of individuals with known relationship, and that reduces to the standard estimator when all individuals are non-inbred and unrelated. Applying our estimator to data simulated based on allele frequencies observed for microsatellite loci in human populations, we find that the new estimator performs favorably compared to the standard estimator, in terms of both bias and mean squared error. For human population-genetic data, we find that a close linear relationship observed between gene diversity and distance from East Africa is preserved when adjusting for the inclusion of close relatives.

GENOME-WIDE SCAN. A NEW GENE OF SUSCEPTIBILITY IN CHILDHOOD ONSET SYSTEMIC LUPUS ERYTHEMATOSUS IN MESTIZO-MEXICAN PATIENTS. *R. Velázquez-Cruz[1], H. García-Ortiz[1], L. Uribe-Figueroa[1], F. Espinosa-Rosales[2], V. Baca[3], L. Orozco[1]* 1) Investigación, Instituto Nacional de Medicina Genomica, SS; 2) Servicio de Inmunología, Instituto Nacional de Pediatría, SS; 3) Hospital de Pediatría Centro Médico Nacional Siglo XXI, IMSS. Mexico City, MEXICO.

Systemic lupus erythematosus (SLE) is a complex illness, prototype of autoimmune diseases that is characterized by production of autoantibodies and multi-organ manifestations. Only 15-17% of all SLE patients has an onset before the 16 years old. In this work, 100 female SLE patients less than 16 years old, which fulfilled the American College of Rheumatology (ACR) criteria for the diagnosis of nephritis and 100 female healthy controls, were recruited from three tertiary medical centers from Mexico City. We performed a genome-wide association scan using the platform Affymetrix 100K SNP mapping array. After applying the filters of quality, 104,890 SNPs were used to the association study. Aside from the expected association between SLE and the HLA region on chromosome 6p21, we found evidence of association in three regions: 1p13.3 (PTPN22), 1q25.1 (TNFSF4, rs10798269) and 2q32 (STAT1, rs1467199). STAT1 was the strongest associated gene to be involved in the susceptibility of the illness. To validate this finding we used TaqMan assay to carry out an association study in an independent group of 230 cases and 300 controls. The SNP rs1467199 was associated with the susceptibility of SLE, also when we used this last methodology (P=0.010; OR =1.46, 95%CI: 1.09-1.97). Furthermore, when the sample was stratified by gender, distribution of the STAT1 SNP revealed a stronger association with female patients (P=0.0011; OR=1.72, 95%CI: 1.23-2.39). Our preliminary results suggest that there are important differences between these new regions associated with SLE in Mestizo-Mexican and those reported in other populations. Further studies are necessary with samples of different populations to validate our results.

Analysis of East Asia Genetic Substructure: Population Differentiation and PCA Clusters Correlate with Geographic Distribution. *C. Tian[1], R. Kosoy[1], A. Lee[2], P. Gregersen[2], J. Belmont[2], M. Seldin[1]* 1) Rowe Program Human Genetics, Univ California Sch Medicine, Davis, CA; 2) North Shore-LIJ Res Inst, Manhasset, NY, Baylor Col Med., Houston TX.

Accounting for genetic substructure within European populations has been important in reducing type 1 errors in genetic studies of complex disease. As efforts to understand complex genetic disease are expanded to other continental populations an understanding of genetic substructure within these continents will be useful in design and execution of association tests. In this study, population differentiation(Fst) and Principal Components Analyses(PCA) are examined using >200K genotypes from multiple populations of East Asian ancestry(total 298 subjects). The population groups included those from the Human Genome Diversity Panel[Cambodian(CAMB), Yi, Daur, Mongolian(MGL), Lahu, Dai, Hezhen, Miaozu, Naxi, Oroqen, She, Tu, Tujia, Naxi, and Xibo], HapMap(CHB and JPT), and East Asian or East Asian American subjects of Vietnamese(VIET), Korean(KOR), Filipino(FIL) and Chinese ancestry. Paired Fst(Wei and Cockerham) showed close relationships between CHB and several large East Asian population groups(CHB/KOR, 0.0019; CHB/JPT, 00651; CHB/VIET, 0.0065) with larger separation with FIL(CHB/FIL, 0.014). Low levels of differentiation were also observed between DAI and VIET(0.0045) and between VIET and CAMB(0.0062). Similarly, small Fsts were observed among different presumed Han Chinese populations originating in different regions of mainland of China and Taiwan(Fst < 0.0025 with CHB). For PCA, the first two PCs showed a pattern of relationships that closely followed the geographic distribution of the different East Asian populations. For example, the four corner groups were JPT, FIL, CAMB and MGL with the CHB forming the center group, and KOR was between CHB and JPT. Other small ethnic groups were also in rough geographic correlation with their putative origins. These studies have also enabled the selection of a subset of East Asian substructure ancestry informative markers(EASTASAIMS) that may be useful for future genetic association studies in reducing type 1 errors and in identifying homogeneous groups.

Relation of Paraoxonase (*PON2*) Polymorphisms with PON Activity and Systemic Lupus Erythematosus (SLE).

S. Dasgupta[1], F. Y. Demirci[1], A. Dressen[1], A. Kao[2], C. Kammerer[1], S. Manzi[2], M. I. Kamboh[1] 1) Dept. Human Genetics, Univ. Pittsburgh, GSPH, Pittsburgh, PA; 2) Lupus Center of Excellence, University of Pittsburgh, Pittsburgh, PA.

SLE, a severe autoimmune disease that predominantly affects women at their child bearing age, is characterized by chronic inflammation which can virtually affect almost every organ in the body. It is now well accepted that inflammation plays a major role in the atherosclerotic process and SLE women at their 35-44 years of age have 50 fold higher risk of premature coronary heart disease (CHD) than in general population. Many studies have reported that low PON activity is associated with increased risk of CHD and our studies have shown that low PON activity is independently associated with SLE. *PON1*, *PON2* and *PON3* are members of the paraoxonase multigene family, clustered on chromosome 7q21.3-22.1. Two nonsynonymous SNPs in the PON1 gene (L55M and Q192R) have been shown to be major regulators of PON activity. The purpose of this study was to determine the impact of *PON2* polymorphims on PON activity and SLE risk. We screened a total of 19 *PON2* tag SNPs as documented in HapMap and SeattleSNP databases in 345 Caucasian SLE women and 454 Caucasian healthy control women, using pyrosequencing, RFLP or TaqMan allelic discrimination methods. One of the SNPs significantly deviated from Hardy-Weinberg equlibrium and hence was excluded from further analysis. Pairwise linkage disequlibruim (LD) and tagger analyses identified 14 tag SNP bins that would capture the entire set of 18 *PON2* SNPs in our sample. None of the 18 SNPs were found to be associated with SLE risk in single site analysis. Seven SNPs were found to be associated (P0.01) with serum PON activity when adjusted for age, BMI and smoking in single site analysis. In a multiple regression model that included also the two *PON1* coding SNPs (L55M and Q192R), only two *PON2* SNPs remained significant (P0.05) that accounted for about 1.5 % of the PON activity variation. These results indicate that in addition to *PON1* SNPs, *PON2* genetic variation also contributes towards serum PON activity although to a much lesser degree.

Acceptance and compassion: prejudicial attitudes to patients bearing genetic disorders. *J. Forbes[1,2], C. Riolfi[1,2], A. Bogochvol[1,2], T. Genesini[1,2], E. Macedo[1,2], M. Zatz[1]* 1) Human Genome Research Center, University of São Paulo (USP), São Paulo, São Paulo, Brazil; 2) Institute of Lacanian Pshychoanalysis (IPLA), São Paulo, São Paulo, Brazil.

The diagnosis of a degenerative condition, such as a neuromuscular disease, tends, at first, to provoke in the patient a reaction of rage or denying of the fact. These subjective behaviors are usually followed by the acceptance of the patient and the compassion of his family. The knowledge of being affected by a progressive untreatable disorder makes them think they have found a destiny to follow. We do not agree with this approach and consider that this is an inappropriate way to deal with this situation. Our hypothesis is that besides the impact on normal relatives, this approach precipitates the patients disability and increases the speed of the diseases progression. In opposition to this established trend, we have treated twenty four patients with different neuromuscular conditions, with a novel psychoanalytical technique, which is based in dis-authorizing the socially standard expected behavior. The patients (7 myotonic dystrophy, 5 limb-girdle muscular dystrophy, 4 cerebelar ataxias, 3 facio-scapulohumeral, 2 Duchenne, 1 Becker, 1 Friedreich's ataxia, 1 congenital muscular dystrophy) were seen in a weekly basis by a trained team of supervised psychoanalysts. Preliminary results were discussed by the group and documented in video. After three months, we observed a significant modification in patients behavior. We were able to witness the replacement of acceptance by invention; and of compassion for shared responsibility. The results are surprising, and in addition to the patients they are causing a great impact in their families and in the involved clinical team.

Exploratory Analyses for Positive Natural Selection in Amerindian Populations. *R. Kosoy[1], C. Tian[1], M. Ransom[1], W. C. Knowler[2], R. L. Hanson[2], G. Silva[3], J. W. Belmont[4], M. F. Seldin[1]* 1) UC Davis, Davis, CA; 2) NIDDK, NIH, Phoenix, AZ; 3) Obras Sociales del Hermano Pedro, Antigua, Guatemala; 4) Baylor Col Med., Houston, TX.

Studies in European, East Asian (EAS) and African populations have provided evidence for the role of natural selection in the evolution of their genomes. Other evidence suggests that some positively selected alleles are paradoxically associated with adverse effects in modern populations. In order to identify genetic variants that might predispose to major medical problems (type 2 diabetes (T2D) and rheumatoid arthritis (RA)) in Amerindians (AMI) we are exploring for evidence of positive selection unique to AMI group(s). Information from genome scans (Illumina 300K SNP) in 23 Pima and 24 Maya AMI and public HapMap data from EAS were analyzed using three approaches: 1) population differentiation as measured by AMI/EAS Fst value distribution; 2) allele frequency spectrum analyses using composite likelihood ratio test (CLR); and 3) integrated haplotype analyses (iHS) for detection of recent selective sweeps. The distribution of the significant signals detected by each of the methods is nonrandom, suggesting multiple loci subject to positive selection. First, 127 SNPs with Fst > 0.6 in twenty-seven 300 kb segments (7.8 x106 bp) contrasts 652 SNPs with Fst >0.6 present in the rest of the genome (~2.7 x 109 bp). For allele frequency spectrum analyses a total of 168 autosomal locations showed CLR p values of <0.002 (p values determined by neutral model coalescent simulations). Of these 69 were specific for AMI and 53 for EAS suggesting strong differences in selection in these populations. Similarly several loci were suggested by iHS methods. Notably, some of the loci were identified by both Fst and CLR tests. Also, of particular note were three genes located within 100 kb of the putative selection peaks: taste receptor, type 1, member 1 (TAS1R1), melanin-concentrating hormone receptor 1 (MCHR1) and CD86. The first two may play a role in predisposition to T2D, and CD86, a ligand for the CD28 T cell co-receptor, may be a potential candidate for increased prevalence of RA in AMI populations.

Genome-wide linkage and association scans for quantitative trait loci of serum lactate dehydrogenase -- the Framingham Heart Study. *J. Lin[1], A. Cupples[2], G. Zheng[1], J. Joo[1], C. O'Donnell[3]* 1) Office of Biostatistics Research/DPPS/NHLBI/NIH Bethesda, MD; 2) Department of Biostatistics, Boston Univeristy, Boston, MA; 3) Framingham Heart Study/NHLBI/NIH, Boston, MA.

Serum lactate dehydrogenase (LDH) is used in diagnosing heart, liver, and muscle diseases, especially to help monitor a heart attack. Family studies have reported that serum LDH variation is significantly determined by genetic factors with heritability estimates approximately 50%. Three genes coding for LDH isoenzymes were mapped to chromosome 11q15 and 12p12. To date, no linkage or association analysis on serum LDH levels has been reported. We carried out a 10 cM microsatellite genome-wide linkage scan and a 100K SNP genome-wide association scan for quantitative trait loci of LDH in a community-based Caucasian cohort, the Framingham Heart Study. Our study population consisted of 330 families with 1702 individuals being microsatellite genotyped and 1343 individuals SNP genotyped. Using variance-component linkage methods, the heritability was estimated at 41%. The genome-wide linkage analysis yielded several chromosomal regions with LOD scores between 1 and 2.5 on chromosomes 3, 4, 7, 8, 9, 19 and 20, respectively. The 100K SNP genome-wide association study did not identify any SNP with genome-wide significance ($p<10-7$). None of the 32 SNPs with a p-value<10-4 was close to the chromosomal regions where the LDH genes reside or within the linkage peaks. Our study demonstrated a strong genetic effect on the variation of LDH levels. Instead of a single gene with a large effect, there may be several genes with small effects in controlling the variation of serum LDH. Those genes appear to be located on chromosomal regions that differ from where the genes encoding LDH isoenzymes reside.

Genotype and tissue effects on alternative splicing of the TCF7L2 gene in tissues important to Type2 Diabetes(T2DM)pathogenesis. *A. Mondal[1, 2], S. Das[1, 2], W. Chu[1, 2], N. Sharma[1, 2], S. Elbein[1, 2]* 1) University of Arkansas for Medical Sciences; 2) CAVHS, Little Rock, AR.

SNPs of the TCF7L2 gene are the strongest genetic risk factors for T2DM,but the variants are intronic and of unknown function.TCF7L2 has multiple alternative splice forms,but no data are available on this in tissues playing key role in T2DM pathogenesis. We hypothesized that TCF7L2 SNPs altered T2DM risk by changing expression of alternative splice forms in human tissues involved in T2DM pathophysiology.We isolated total RNA from human subcutaneous (sc)adipose and muscle biopsy samples,HepG2 cells and a commercial total pancreas RNA sample to identify all TCF7L2 splice forms using 5and3RACE technique and PCR based cloning. We quantified total and transcript isoform specific TCF7L2 levels in sc.adipose of 78 healthy non diabetic human subjects who were genotyped for SNPs rs7903146 and rs12255372 and who represented a wide range of BMI and S_I.In adipose we found two alternate 3ends of TCF7L2 mRNA, one isoform being 262bp shorter then the other.The shorter isoform was not observed in muscle.We confirmed expression in all tissues of a previously reported 69bp alternatively spliced exon within intron3(exon 3a),and identified a novel 141bp exon within intron4(exon 4a) expressed only in muscle tissue and HepG2 cells. In muscle,we also observed a novel 235bp exon formed by alternative splicing of parts of exons3 and4.A 73bp alternatively spliced exon from within intron13(13a) was present in all tissues and was present the most abundant isoform in the pancreas. We observed 16 different TCF7L2 alternatively spliced transcripts.Based on screening >80 clones each in adipose,muscle, pancreas and HepG2 cells, we identified 8,14,8 and 10 isoforms,respectively.Whereas total TCF7L2 levels in sc.adipose were not correlated with BMI or S_I,transcripts retaining exon 13a in adipose were significantly correlated with BMI (p=0.01)and %fat(p=0.002).Furthermore,exon13a containing transcripts were significantly associated with genotype at SNP rs7903146(p=0.03). These studies suggest that TCF7L2 intronic SNPs may alter alternative splicing or transcript-specific stability of TCF7L2 in human adipose.

Y chromosomal short tandem repeats (Y-STRs) and its association with azoospermia factors (AZF) microdeletions of infertile males. *L. Pliss[1], I. Pelnena[1], A. Puzuka[2], A. Sabule[3], S. Rozane[3], A. Ivanovs[2,3], V. Baumanis[1], A. Krumina[2]* 1) Latvian Biomedical Research and Study Centre, Riga, Latvia; 2) Riga Stradins University, Riga, Latvia; 3) Latvia State Centre for Forensic Medical Examination, Riga, Latvia.

Introduction.A series of polymorphic Y-chromosomal short tandem repeats (Y-STRs) show high levels of heterogeneity and thus provide a useful tool for population studies as well as for searching association with so called the infertility haplotype. The most common genetic cause of human Y-linked male infertility is partial or complete deletions of the AZF region on the Y chromosome. The aim of the present study was to characterize 12 Y-STRs haplotype variation in infertile males that possess AZF region microdeletions. Materials. Objects for studies of Y-STRs variation and its association with male infertility were five individuals with different spermatogenic arrest. Methods. 12 Y-STRs were determined by PowerPlex Y System (Promega, USA). Microdeletions in AZF region were determined by two multiplex PCR amplifications using ten primer pairs. Internal PCR control (ZFX/ZFY) was used as well as DNA sample from a fertile male and female. Results. Out of five analyzed samples, we have found 2 cases with microdeletions that were observed in three AZF regions at SY86 and SY84 (AZFa), SY127 and SY134 (AZFb), and SY254 and SY255 loci in DAZ gene family (AZFc). 3 individuals showed Y chromosomal microdeletions only in AZFc region. Two individuals with AZFc region microdeletions belonged to the most frequent haplogroup among Baltic Sea region populations, N3a1, and Y-STRs haplotypes of these samples were almost identical. For two samples with complete AZF region microdeletions, most of Y-STRs that are localized in the long arm of Y chromosome were deleted except DYS438 and DYS392. Y-STRs from the short arm of Y chromosome have been amplified, and the following alleles were observed: 15 (DYS19) and 13/14 (DYS393). Conclusion. Use of Y-STRs provides the precise location of AZF region microdeletions in comparison with non-polymorphic STS loci and points on the presence or lack of Y-chromosome arms (isochromosome).

Pre-symptomatic risk assessment for complex genetic diseases. *B. Padhukasahasram, E. Halperin, J. Wessel, D. Thomas, E. Silver, H. Trumbower, N. J. Schork, M. Cargill, D. A. Stephan* Navigenics Inc., Redwood Shores, CA, U.S.A. 94065.

The prevalence of common chronic non-communicable diseases (CCND) far overshadows the prevalence of both monogenic and infectious diseases combined. Common SNP variants account for a substantial portion of the genetic risk for such diseases and when used in context will allow for more personalized and focused exposure mitigation, early detection, and early intervention paradigms. We present a novel measure called the genetic composite index (GCI), which considers only summary statistics of the effects of common genetic variants and show its predictive power as a clinical classifier. Predictive power of the GCI varies by condition and when combined with environmental data should provide an additional tool for clinical decision-making. It will also facilitate prospective studies testing whether actions based on the knowledge of genetic load lead to significant medical benefits and whether this knowledge motivates individuals to adopt healthier lifestyles.

A new case of Cranio-Lenticulo-Sutural dysplasia. *S. Boyadjiev Boyd[1], C. Nauta[1], A. Hata[1], C. Naydenov[2], E. Zackai[3], J. Kim[1]* 1) Dept Pediatrics, Section of Genetics, UCD Med Ctr, Sacramento, CA; 2) Department of Chemistry and Biochemistry, Medical University Sofia, 1431 Sofia, Bulgaria; 3) Division of Human Genetics, Department of Pediatrics, The Children's Hospital of Philadelphia, Philadelphia, Pennsylvania.

We delineated Cranio-lenticulo-sutural dysplasia (CLSD; OMIM 607812, Boyadjiev-Jabs syndrome) as a new autosomal recessive syndrome in a consanguineous family where five males and one female have distinctive craniofacial features (large and late-closing fontanels, hypertelorism), early onset cataracts, and mild generalized skeletal dysplasia. Linkage analysis mapped the locus to chr. 14q13-q21 and a F382L mutation was identified in SEC23A. Detailed molecular and biochemical analysis of wild type and mutant SEC23A, an integral member of the COPII-mediated ER-to-Golgi trafficking pathway, led to better characterization of intracellular trafficking in health and disease. A zebrafish morpholino model recapitulated the human phenotype. Recently, an unrelated individual with clinical features consistent with CLSD was identified. Molecular analysis of SEC23A identified a novel heterozygous M702V SEC23A mutation involving a highly conserved residue. This missense mutation was inherited from the unaffected father and was not present in 400 control chromosomes. No mutations were found in the maternal alleles and SEC23A real-time PCR analysis showed normal expression of both alleles. Biochemical characterization by in vitro COPII vesicle budding assay documented that the M702V mutation results in impaired COPII vesicle formation. Our data suggest that CLSD may be more common than previously thought and should be considered in the evaluation of patients with late-closing fontanels. Alternative inheritance patterns may exist for this syndrome.

EDA signaling is required for sweat gland development and hair subtype formation. *C. Cui, M. Kunisada, D. Esibizione, E. Douglass, D. Schlessinger* Laboratory Genetics, NIA/NIH, Baltimore, MD.

EDA signaling is pivotal in the initiation of skin appendages. Its possible involvement in appendage subtype determination and post-induction stage appendage development, however, has not been studied systematically. To address these issues we manipulated conditional expression of an Eda-A1 transgene in Tabby mice, where the transgene is the only source of active ectodysplasin (Eda). We find that Eda-A1 restores sweat glands and all hair subtypes in Tabby, but each require its action at an idiosyncratic time of development: by E17 for guard, by E19 for awl, and starting at E18 for zigzag/auchen hair. Guard and awl hairs were indistinguishable from their wild-type counterparts; but restored zigzag and auchen hairs, while recognizable, were somewhat smaller and lacked characteristic bends. Notably, secondary hair follicle formation of awl, auchen, and zigzag hairs required higher Eda-A1 expression level than did guard hair or sweat glands. Furthermore, Eda-A1 expression is required until the early dermal papilla stage for guard hair germs to make follicles, but is dispensable for their maturation. Similarly, sweat gland pegs require Eda-A1 at an early stage to form mature glands. Thus we infer that Eda signaling is needed for the determination and development of various skin appendages at spatiotemporally restricted intervals.

A novel ERG rearrangement in acute myeloid leukemia with t(16;21)(p11;q22) and t(15;17)(q22;q21) translocations. *S. Hazourli, J. Hébert* Quebec Leukemia Cell Bank, Maisonneuve-Rosemont Hospital Research center, Montreal, PQ, Canada.

Cytogenetic and molecular studies have established a correlation between acute promyelocytic leukemia (APL) and the presence of the specific t(15;17)(q22;q21) translocation leading to the PML-RARA fusion gene. In APL patients, the t(15;17) predicts a favourable differentiation response to all-trans retinoic acid (ATRA) and a very good survival. We here report a unique case of immature acute myeloid leukemia (AML) with basophilia, presenting a t(16;21)(p11;q22) translocation and an additional t(15;17)(q22;q21) translocation identical to the APL translocation by G-banding. The presence of the t(15;17) translocation as a secondary event and in non-promyelocytic leukemia, is unusual. In our case, FISH analysis with a PML-RARA dual color probe revealed one fusion signal on the derivative chromosome 15, suggesting a PML-RARA positive fusion. However, the second fusion signal was absent and no other RARA rearrangement was detected using a breakapart RARA probe. RT-PCR analysis confirmed the absence of PML-RARA fusion transcripts. These results suggest that the chromosomal breakpoints are located in the vicinity of the PML and RARA genes. The t(16;21) translocation is a rare event in AML, in myelodysplastic syndrome and in the blastic phase of chronic myelogenous leukemia, conferring a poor prognosis. This translocation fused the FUS gene on chromosome 16 to a member of the ETS transcription factor (ERG) on chromosome 21, generating an oncogenic FUS-ERG fusion, also found in Ewing's tumours. In our case, molecular cytogenetics investigation of the t(16;21)(p11;q22) chromosomal breakpoints with BAC probes, confirmed the involvement of the ERG oncogene on the chromosomal band 21q22. However, the translocation breakpoint on 16p11.2 was centromeric to the FUS gene. To our knowledge, this is the first case of AML showing a t(16;21) translocation associated with a t(15;17). The t(15;17) in this case was molecularly different from the APL translocation. Furthermore, a potential novel ERG fusion gene was identified in this leukemia.

Reversal of glycogen storage disease type III related cardiomyopathy with high protein diet. *A. Dagli[1], R. Zori[1], H. McCune[1], T. Ivsic[3], M. Maisenbacher[1], D. Weinstein[2]* 1) Dept Peds, Div Gen & Met, Univ Florida, Gainesville, FL; 2) Dept Peds, Div of Endocrinology, Univ Florida, Gainesville, FL; 3) Dept Peds, Div of Cardiology, Univ Florida, Gainesville, FL.

Glycogen storage disease type III is caused by a deficiency of glycogen debranching enzyme leading to impaired release of glucose from glycogen. Accumulation of glycogen with short outer chains occurs. Most patients (85%) have muscle and liver involvement (GSD IIIa). These individuals have hypoglycemia, hyperlipidemia and hepatomegaly with elevated transaminases in childhood. In adolescence and adulthood, progressive muscle weakness and hypertrophic cardiomyopathy can be predominant features. The management consists of cornstarch supplementation and high protein diet. Conventionally, it is believed that this management is effective in preventing hypoglycemia but does not prevent or improve progressive myopathy or cardiomyopathy. Slonim et al (1981) reported marked improvement in muscle strength and mass after initiation of a high protein diet in a child with GSD III. There has been little discussion regarding the effect of high protein intake on myopathy and cardiomyopathy since this case report.

We report a 22 year old male with GSD IIIa diagnosed with severe cardiomyopathy at 14 years of age. A high protein intake of 2g/kg was recommended with no improvement in the cardiomyopathy. For the past one and a half years, however, we increased the protein intake to 3g/kg/day combined with restriction of simple carbohydrates. There was significant reversal of cardiomyopathy as seen on echocardiography, with left ventricular mass improving from 284g to 143g and left ventricular mass index improving from 160g/m2 to 78g/m2 in a span of one year. With this case we would like to emphasize the role of high protein intake in patients with GSD III and cardiomyopathy.

Studying genetic contributions by mother and child to susceptibility: efficient design and analysis for diseases of early life. *M. Shi[1], D. M. Umbach[1], S. H. Vermeulen[2], C. R. Weinberg[1]* 1) Biostatistics Br, NIEHS, Res Triangle Park, NC; 2) Department of Endocrinology, Department of Epidemiology, Biostatistics and HTA (133) & Department of Human Genetics, Radboud University Nijmegen Medical Centre, PO Box 9101, 6500 HB Nijmegen, The Netherlands.

The prenatal environment can play an important role in the etiology of disease, particularly for conditions with onset early in life, such as childhood cancers and birth defects. To capture the potential effects on risk from both maternal and fetal genotypes, investigators can use designs that require genotyping of affected offspring and their mothers , e.g. the population-based case-mother/control-mother design or the hybrid case-parent triad/controlparents design. For the case-mother/control-mother design, the usual logistic regression analysis fails to fully exploit the fact that the pairs are mothers and their offspring. We show that by enforcing three natural and plausible nested levels of assumptions (i.e. Mendelian inheritance, mating symmetry, and parental allelic exchangeability) we can improve the efficiency of this design. Calculations reveal that these improvements over the usual logistic analysis can be substantial, even if only the Mendelian assumption is honored. We also consider an alternative hybrid design where offspring-mother dyads, instead of parents, serve as population-based controls, a design that is especially valuable when control fathers are hard to recruit. We show that this design retains the ability to test for mating symmetry and absence of population stratification bias and can be used to estimate offspring and maternal genotypic effects even when these assumptions do not hold, but with reduced power. We compare the power of the alternative hybrid design and the case-mother/control-mother design with the previously described case-parent triad and hybrid with control parents designs, and show that the two proposed designs perform well in certain scenarios.

RUNX1-USP42 is a recurrent fusion gene in acute myeloid leukemia. *A. Giguère[1], P. Chagnon[2], J. Hébert[1,2]* 1) Quebec Leukemia Cell Bank, Maisonneuve-Rosemont Hospital Research center; 2) Institute for Research in Immunology and Cancer, University of Montreal.

The RUNX1 gene encodes a transcription factor essential for the development of definitive hematopoiesis. In acute leukemia, RUNX1 is frequently rearranged with several partner genes in chromosomal translocations. Recently, a new cryptic t(7;21) translocation involving RUNX1 and a deubiquitinating enzyme, USP42 was described in a childhood acute myeloid leukemia (AML). USP42 belongs to the ubiquitin-specific peptidase (USP) family which shares highly conserved regions of amino acid sequence that form the catalytic domain of these enzymes. We describe the identification of USP42 as a recurrent partner gene of RUNX1 in leukemic cells of an adult patient with AML. The karyotype was 46,XY,del(5)(q22q33),?del(21)(q22). Spectral karyotyping and FISH with BAC probes revealed a cryptic t(7;21)(p22;q22) translocation involving RUNX1 and USP42. An in-frame fusion between exon 7 of RUNX1 and exon 3 of USP42 and an alternative transcript with a deletion of RUNX1 exon 6 were confirmed by RT-PCR and sequencing. A 10-fold upregulation of USP42 mRNA was detected in the t(7;21) leukemic cells by quantitative RT-PCR. No other t(7;21) positive case was detected in a RT-PCR study of 109 samples of myeloid leukemia. Sequencing of genomic DNA and bioinformatics analysis revealed a 7bp microhomology near the translocation breakpoint and an in vitro topoisomerase II consensus cleavage site in intron 2 of USP42. A non-homologous end-joining repair mechanism may be involved in the generation of this fusion. By immunofluorescence, we demonstrated a nuclear expression of the chimeric protein suggesting that RUNX1-USP42 might inhibit RUNX1-mediated transactivation, a mechanism of leukemogenesis previously described in other RUNX1 fusion proteins. We are currently evaluating the expression of different known RUNX1 target genes in the leukemic cells positive for RUNX1-USP42, in order to better understand the oncogenic potential of this fusion. USP42 is part of a novel class of genes involved in the pathogenesis of leukemia and its role in hematologic cancers remains to be studied.

Four novel mutations of the *Italic Text*IRF6*Italic Text* gene in Thai families with Van der Woude syndrome. *K. Suphapeetiporn, P. Yeetong, V. Shotelersuk* Division of Medical Genetics and Metabolism, Department of Pediatrics, Chulalongkorn University, Bangkok, Thailand.

Van der Woude syndrome (VWS) is a dominantly inherited disorder characterized by cleft lip with or without cleft palate and lip pits. It remains the most common syndromic form of oral clefts. Mutations in the *Italic Text*interferon regulatory factor 6 gene*Italic Text* (*Italic Text*IRF6*Italic Text*) have been identified in patients with Van der Woude syndrome. Its mutations also result in non-syndromic cleft lip with or without cleft palate in some populations. We identified four unrelated Thai families with VWS and performed mutation analysis by PCR-sequencing the entire coding region of the *Italic Text*IRF6*Italic Text* gene. Four potentially pathogenic mutations, c.145CT (p.Q49X), c.171TG (p.F57L), 1306CG (p.L436V), and IVS3-3CG were successfully detected in four unrelated patients. All these mutations were not detected in at least 100 unaffected ethnic-matched control chromosomes and have never been previously reported. The p.Q49X and p.F57L mutations were located in the highly conserved DNA binding domain while the p.L436V was located at the most carboxy-terminal region ever reported. This study demonstrated that several distinct mutations of *Italic Text*IRF6*Italic Text* occurred in the Thai families with VWS and thus expanded the spectrum of the *Italic Text*IRF6*Italic Text* gene mutations.

State Space Reduction in Hidden Markov Model for Haplotyping, Imputation and Analysis of Shotgun Sequence Data. *W. Chen, Y. Li, G. R. Abecasis* Center for statistical Genetics, Department of Biostatistics, University of Michigan, Ann Arbor, MI.

The size of datasets utilized for gene-mapping studies to access the human genetic variation is rapidly increasing. It is now common to analyze genome-wide association studies (GWAS) with information on 100,000s of SNPs for 1000s of individuals and whole genome shotgun sequencing is rapidly becoming feasible. It is now clear that imputation of missing genotypes can improve power for GWAS and attempts are now underway to combine 'low-depth' shotgun sequence data across many individuals. However, accurate estimation of haplotypes and missing genotypes in these large datasets remains an important but challenging computational problem. Here, we describe a series of computational enhancements for hidden Markov models (HMM) that describe the haplotypes in each individual as an imperfect mosaic of other sampled haplotypes and which are a key component of several algorithms for genotype imputation and analysis of shotgun sequence data. Our enhancements take advantage of similarity among haplotype segments between individuals to reduce the number of distinct states that must be modeled in the HMM and results in no loss of accuracy. Simulation and analysis of real datasets show that our method can reduce computing time by a factor of 2-5x and reduce memory usage by a factor of 6-16x compared to current implementations of these HMMs. We hope this algorithm will be a useful addition to the toolbox of human geneticists.

Sapropterin (Kuvan) is safe and effective in patients under 4 years of age with phenylketonuria (PKU). *D. Hartung[1,2], H. Bausell[1], R. Katz[1], B. K. Burton[1,2]* 1) Dept Genetics, Children's Memorial Hosp, Chicago, IL; 2) Feinberg School of Medicine, Northwestern University, Chicago, IL.

In December, 2007, Kuvan was approved by the FDA for use in patients with hyperphenylalaninemia due to tetrahydrobiopterin(BH4)-responsive PKU. Clinical trials of Kuvan did not include children under 4 years of age. Since FDA approval, 11 children under 4 years of age (range 7 mo- 4 yrs) with PKU have been treated with Kuvan in the PKU Clinic at Children's Memorial Hospital in Chicago. All were well-controlled with mean blood phenylalanine (phe) levels below 360 umol/L at the time of initiation of drug therapy. Blood phe levels and diet records were obtained at baseline, 24 hrs, 1 wk and 2 wks; the dose used was 20 mg/kg/day given in apple juice. Response was defined as a decline in the blood phe level of 30% below baseline. 7 pts were responders; 2 non-responders and 2 not yet determined. The mean decline in blood phe among responders was 58% (range 32-74). One pt. experienced diarrhea when drug was initiated; this resolved within one wk. on continued treatment. No other adverse events were reported. Total length of time on therapy for all patients ranged from 1-5 months. A 2 yo responsive pt on a medical food (formula) was able to discontinue this and is now on an unrestricted diet. Two infants with mild PKU under one year of age were started on Kuvan without dietary restrictions when their blood phe levels reached a threshold requiring intervention. They remain on unrestricted diets with blood phe levels in the near normal range. The other 4 responsive patients have had their diets liberalized to varying degrees while maintaining excellent blood phe control. We conclude that Kuvan can be safely and effectively used in children under 4 years of age. Significant diet liberalization can be achieved in many patients without sacrificing blood phe control. In some patients, Kuvan alone can provide excellent blood phe control without the need to institute dietary restrictions.

Association of Variants in the -endomannosidase (*MANEA*) Gene with Cocaine-related Behaviors. *L. A. Farrer[1], H. R. Kranzler[2], Y. Yu[1], R. D. Weiss[3], K. T. Brady[4], J. F. Cubells[5], J. Gelernter[6]* 1) Genetics Program, Boston Univ Sch Medicine, Boston, MA; 2) Department of Psychiatry, Univ Connecticut Sch Medicine, Farmington, CT; 3) Alcohol and Drug Abuse Treatment Program, McLean Hospital, Belmont, MA; 4) Department of Psychiatry and Behavioral Sciences, Medical Univ South Carolina, Charleston, SC; 5) Department of Human Genetics, mory Univ Sch Medicine, Atlanta, GA; 6) Department of Psychiatry, Yale Univ Sch Medicine, New Haven, CT.

Cocaine dependence (CD) and related behaviors are highly heritable, but no genetic associations have been consistently demonstrated. Our recent genome-wide study identified association of cocaine-induced paranoia (CIP) with a SNP in the -endomannosidase (*MANEA*) gene in European American (EA) and African American (AA) family-based samples enrolled in a genetic study of drug dependence. To pursue this finding, we conducted a comprehensive genetic association study of *MANEA* with CD and CIP. A total of 3,992 individuals from two family-based samples (EA and AA) and two case-control samples (EA and AA) were classified as CD, CIP or control using the Semi-structured Assessment for Drug Dependence and Alcoholism and genotyped for 11 SNPs spanning *MANEA* and its surrounding region. Association of CD and CIP with individual SNPs and haplotypes was evaluated using family-based and population-based methods. CIP was associated with six SNPs in the EA families and nine SNPs in the AA families. The strongest evidence in the total sample of families was observed with 3 markers located in the promoter and 3 UTR regions ($0.000004 < p < 0.00005$). The association of *MANEA* SNPs with CD in both family samples was much weaker. In the AA case-control sample, multiple markers were significantly associated with CIP and CD. CIP and CD were also significantly associated with a two-SNP haplotype in the EA case-control sample. The *A* allele of the 3 UTR SNP rs9387522 was associated with increased risk of CIP in all four datasets. Our findings suggest that CD and associated behaviors may involve biological pathways not typically thought to be associated with brain metabolism.

Genetic Polymorphisms of Glutathione-S-Transferase P1, T1 and M1 in Pediatric Patients with Acute Lymphocytic Leukemia in a Philippine Tertiary Hospital. *C. L. T. Silao[1,2], M. M. L. B. Alcausin[1], P. D. Fajardo[3], A. Goleta-Dy[3], E. Melendres[3], E. M. dela Paz[1,2], C. D. Padilla[1,2]* 1) Section of Genetics, Department of Pediatrics, University of the Philippines-Philippine General Hospital; 2) Institute of Human Genetics, National Institutes of Health Philippines; 3) Section of Hematology and Oncology, Department of Pediatrics, University of the Philippines-Philippine General Hospital.

Researches on cancer are geared towards identifying genes and other host factors that may increase or decrease susceptibility risk. Glutathione-S-transferases (GSTs) are major detoxifying polymorphic enzymes that modify susceptibility in various cancers including acute lymphocytic leukemia (ALL). ALL is the most common form of malignancy in childhood with its incidence varying from race to race. This paper determines the frequency of Glutathione-S-transferase polymorphisms (P1, T1, and M1) in Filipino pediatric patients with ALL and in a pediatric control population. It also compares the frequencies between the two groups. All pediatric patients with ALL undergoing treatment at the UP-PGH Medical Center from January to June 2007 were enrolled. Subjects without ALL from the UP-PGH Outpatient Department, age and sex matched, were included as controls. Genomic DNA was extracted from peripheral blood of each subject. Determination of GSTM1 and T1 polymorphisms were done using polymerase chain reaction while restriction fragment length polymorphism analysis was employed for the GSTP1 polymorphism. Comparison between the genomic frequencies of control and ALL patients was done using Matched Odds Ratio. Statistical analysis showed a trend towards protection from having ALL with the presence of GSTT1 and GSTM1 polymorphisms, with OR 0.59 (95% CI: 0.24-1.36) and OR 0.86 (95%CI: 0.36-2.00), respectively. Having the GSTP1 polymorphism, however, was shown to be a risk factor to having ALL with OR 1.7 (95% CI: 0.74-4.15). Differences in the frequencies of GST polymorphisms were noted between the control group and patients with ALL. GSTT1 and GSTM1 polymorphisms appear to be protective from having ALL while having the GSTP1 polymorphism confers increased risk for ALL.

Association of FMR1 repeat size and verbal intelligence quotient (VIQ) independent of FXTAS-associated motor symptoms. *E. G. Allen[1], A. Abramowitz[2], K. Charen[1], D. Hamilton[1], M. Leslie[1], R. Letz[3], G. Novak[1], M. Rusin[4], L. Shubeck[1], J. L. Juncos[5], S. L. Sherman[1]* 1) Dept Human Genetics, Emory Univ, Atlanta, GA; 2) Dept Psychology, Emory Univ, Atlanta, GA; 3) School of Public Health, Emory Univ, Atlanta, GA; 4) Independent Practice, Atlanta, GA; 5) Emory Univ School of Med, Dept Neurology, Atlanta, GA.

A CGG repeat sequence located in the 5 untranslated region of the FMR1 gene when expanded leads to fragile X spectrum disorders. When greater than 200 repeats, the gene is silenced due to hypermethylation, and the lack of protein leads to fragile X syndrome (FXS). Alleles with 55-200 unmethylated repeats, termed premutation alleles, have been associated with ovarian insufficiency (FXPOI) and a late-onset tremor/ataxia syndrome (FXTAS). Several groups have reported neuropsychological deficits among carriers of the premutation, particularly among males with FXTAS. We have previously shown a subtle but significant effect of FMR1 repeat size on verbal intelligence quotient (VIQ) as measured by the Wechsler Adult Intelligence Scale - III (WAIS-III) among 216 females ages 18-50 years. Now, we have confirmed these findings in a study sample of 202 males (n with premutation=84) and 527 females (n with premutation=323) ages 18-85. All subjects completed a neuropsychological test battery including WAIS-III. Repeat size, as a continuous variable, explained 2% of the variation in VIQ based on all study subjects when the model was adjusted for age, race, education, and gender (p-value for repeat size =0.0002). Among 65 males over the age of 50 years (n with premutation =51), the results were more striking: repeat size explained 26% of the variation in VIQ adjusting for the sample covariates (p-value for repeat size <0.0001). Surprisingly, the presence of motor symptoms (tremor or ataxia) was not significant in this model. This suggests that the observed decrease in VIQ is independent of motor symptoms of FXTAS. These findings, including individual WAIS-III subtests, will be presented.

The perception of the university student: genetics, bio-technology and future biomedical applications at a university in Bogota, Colombia. *F. Suarez, A. Ordonez* Instituto de Genética Humana, Universidad Javeriana, Bogota, Colombia.

OBJECTIVE: This project aims to describe the perception, knowledge and attitudes about genetics and its biomedical applications, in a group of university students, as well as determining the degree of information about genetic studies in humans and their uses in research and health services. METHODS: We conducted a qualitative study with university students using 5 focus groups with 5 members each (25 students) and 10 individual semi-structured interviews. The sessions were digitally recorded and a moderator was guiding the group through a discussion around five specific subjects: eugenics, homosexuality, intelligence, bio-technology and transgenic food and their possible relations with biomedical applications, research, and diagnostic techniques. The subjects were students of different areas of knowledge including, engineering, nutrition, psychology, philosophy and theology, all of the students were in their second or third year at the University. The interviews were conducted at one of the main Universities in the capital City Bogotá - Colombia. The analysis of the recordings was conducted using the Atlasti software 5.0 to extract and systematically analyze complex phenomena (students viewpoints) embedded in the narratives from the focal group sessions and interviews. RESULTS: The Participants expressed ideas that confuse the meaning of inheritance and the meaning of birth defect, they agreed that homosexuality was a genetic trait and that definitely there must be a greater access to prenatal diagnostic services in order to genetically improve future generations, including features and traits like the intelligence and sexual orientation. However, most of those interviewed found inconvenient discrimination against people based on their genetic traits. In contrast to the agreement with the human genetic improvement, most participants were in disagreement with the idea of mass distribution of genetically modified food as a solution to the actual alimentary crisis. The reasons and consequences of these attitudes are analyzed in the context of an underdeveloped country.

Premenopausal Breast Cancer Care at a Major Referral Center: Opportunities for Improvement. *K. M. Hennan, K. M. Moore, A. F. Wagner* OB/GYN, University of Oklahoma Health Sciences Center, Oklahoma City, OK.

BACKGROUND: Treatment of breast cancer during reproductive years can be difficult given issues of fertility and potential for premature ovarian failure. Also, these women often have family histories of breast and/or ovarian cancer and may require genetic counseling and testing. OBJECTIVE: This study sought to describe the characteristics of premenopausal breast cancer patients and review their perceptions of care, barriers to treatment, and their recollections of genetic and menopausal symptom counseling. METHODS: Surveys were sent to all living, premenopausal women diagnosed with breast cancer at the OU Breast Institute from 2003-06. Demographics were obtained via chart review. Statistics were performed via SAS v9.1. RESULTS: 176 surveys were mailed; 29 were sent to patients >50 and were excluded. Of the remaining 147, 11 had incorrect addresses and 2 were deceased resulting in 134 eligible. 72(55%) were returned. For the entire cohort, 48% had Medicaid, and 49% private insurance. 43% lived in rural, 8% urban and 49% in metro communities. 68% were Caucasian, 11% Hispanic and 10% Black. Genetic Risk: 36 women(50%) reported a family history of breast or ovarian cancer. 53% had affected 1relatives, and 56% had family diagnosed <50yo. Discussions related to genetic and ovarian cancer risks were reported in 49% and 32%. 13% of patients received genetic counseling, and 18% genetic testing. The majority reported that it was not suggested(72%). Insurance type and size of referral community had no significant effect on whether genetic counseling occurred. The size of referral community had no significant effect on receiving genetic testing. CONCLUSIONS: 1)The majority was pleased with and did not perceive barriers to care, but there was 18% who did experience difficulty. 2)Only 49% of patients recalled genetic discussions, and this was unevenly offered by a variety of medical providers. 3)50% reported high risk family histories, which should prompt a genetic counseling referral. A statewide process which streamlines access and referral for genetic counseling and screening would be valuable initiatives for the future Oklahoma Cancer Institute.

Alternate mechanisms for BRCA1 silencing in young women with breast cancer. *E. M. Wong[1], A. Dobrovic[2], L. Smith[1], J. Hopper[3], M. Jenkins[3], A. Tesoriero[1], M. Brown[4], M. Southey[1], Breast Cancer Family Registry* 1) Department of Pathology,The University of Melbourne; 2) Peter MacCallum Cancer Centre; 3) Centre for Molecular, Environmental, Genetic and Analytic Epidemiology,The University of Melbourne; 4) School of Molecular and Microbial Sciences, The University of Queensland.

Breast cancer is the most common carcinoma in women.Identifiable *BRCA1* and *BRCA2* mutations do not explain the majority of early-onset breast cancers with a strong family history.Currently known *BRCA1* mutations explain 10% of early-onset breast cancers and 20% of early-onset breast cancers with a strong family history.A proportion of women without identifiable BRCA1 mutations come from multiple-case families that show linkage to the loci and/or have breast tumour morphology consistent with carrying a *BRCA1* mutation.We therefore hypothesize that there may be additional inactivating mechanisms in *BRCA1* that are yet to be described.Using Multiplex Ligation-dependent Probe Amplification(MLPA),we screened for large genomic alterations in 132 case probands(diagnosed under 40 years) from the Australian Breast Cancer Family Study(ABCFS) with either a strong family history and/or BRCA1-like tumour morphology.We found 5 probands with large deletions in *BRCA1*:two had large deletions of *BRCA1* Exons1A-23 and 3 others had deletions of Exons1A-2,Exon5 and Exon20,respectively.To assess the potential for rare and common genetic variants in the regulatory regions of BRCA1 to be associated with breast cancer risk,we used High-Resolution Melt(HRM) analysis and a case-control study of early-onset breast cancer(ABCFS) to study 7 SNPs within the 3UTR and a highly conserved 116bp region.Twelve SNPs in the 5UTR and promoter region of BRCA1 and two evolutionally conserved regions within Intron2(CNS-1;CNS-2) and *BRCA1* Exon2 were also screened.The SNPs selected for analysis are predicted in silico to affect either the binding of *BRCA1* regulatory elements or to create a secondary structure change in the RNA.We will present the outcomes of our screen for large genomic alterations and regulatory region genetic variants and their association with breast cancer risk.

MicroRNA regulation and the variability of human cortical gene expression. *R. Zhang*[1,2,3], *B. Su*[1,2] 1) State Key Laboratory of Genetic Resources and Evolution, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan, China; 2) Kunming Primate Research Center, Chinese Academy of Sciences, Kunming , Yunnan, China; 3) Graduate School of Chinese Academy of Sciences, Beijing , China.

Understanding the driving forces of gene expression variation within human populations will provide important insights into the molecular basis of human phenotypic variation. In the genome, the gene expression variability is different among genes, and at present, most research has focused on identifying the genetic variants responsible for the within population gene expression variation. However, little is known about whether microRNAs (miRNAs), which are small noncoding RNAs modulating expression of their target genes in the human genome, could have impact on the variability of gene expression. Here we demonstrate that miRNAs likely lead to the difference of expression variability among genes. With the use of the genome-wide expression data in 193 human brain samples, we show that the increased variability of gene expression is concomitant with the increased number of the miRNA seeds interacting with the target genes, suggesting a direct influence of miRNA on gene expression variability. Compared with the non-miRNA-target genes, genes targeted by more than two miRNA seeds have increased expression variability, independent of their functional categories or targeted miRNA types. In addition, SNPs located in the miRNA binding sites could further increase the gene expression variability of the target genes. We propose that miRNAs are one of the driving forces causing expression variability in the human genome, which might eventually contribute to phenotypic variation in humans.

RENAL DYSPLASIA - COARCTATION OF AORTA. A NEW ASSOCIATION/SYNDROME: A REPORT OF 6 CASES. *B. Chung, D. Chitayat* Clinical & Metabolic Genetics, Hospital for Sick Children, Toronto, Ontario, Canada.

Renal dysplasia is a congenital disorder of the kidneys, characterized by undifferentiated mesenchyme, immature collecting tubules and abnormal organization and is nearly always cystic. Aortic coarctation is defined as a narrowing of the aorta in the area where the ductus inserts. It may be seen as an isolated defect or associated with congenital abnormalities of the aortic valve and the left heart. The concurrent findings of aortic coarctation and renal dysplasia have not been described previously. We report a series of 6 cases with aortic coarctation and renal dysplasia with different degree of severity and outcomes. Case series (table): The 6 cases were of mixed ethnic background. Family history of renal problems was noted in 3 of the 6 patients and one case was the product of a consanguineous marriage (first cousins). All but 1 had bilateral multicystic renal dysplasia detected antenatally and only in 2 was the coarctation detected prenatally. One patient had facial features consistent with Potter sequence. Three others had common facial features with a high flat forehead, large fontanelles, deep-set eyes, hypertelorism, short noses, slightly low-set ears, single palmer creases and deep-set nails. There was no evidence of severe intrauterine growth retardation or other major birth defects. All had normal karyotypes (patient 3 had a familial inversion of chromosome 18 inherited from his mother) and no deletion at 22q11.2. One pregnancy was terminated, one child died at 3 days of life, one survived and has end-stage renal failure and mild-to-moderate developmental delay and the other 3 survived without major issues following repair of the coarctation. The combination of renal dysplasia and congenital heart defects is rare and has been reported in a variety of chromosome abnormalities, single gene disorders (Meckel syndrome) and VACTERL association. However, the combination of renal dysplasia and coarctation of aorta is a rare event and can represent a hitherto new condition with yet unknown mode of inheritance. Parental consanguinity in one of our cases suggests autosomal recessive mode of inheritance.

ART3 is a novel risk gene for Alzheimer disease. *H. D. Yamagata[1], Z. Zhang[2], W. Zhong[2], R. Hata[3], H. Akatsu[4], K. Kamino[5], M. Takeda[5], K. Kosaka[4], T. Miki[2]* 1) Dept Preventive Medicine, Ehime University Graduate School of Medicine, Toon, Ehime, Japan; 2) Dept Geriatric Medicine, Ehime Univ Grad Sch Med, Toon, Ehime, Japan; 3) Dept Functional Histology, Ehime Univ Grad Sch Med, Toon, Ehime, Japan; 4) Choju Medical Institute, Fukushimura Hospital, Toyohashi, Japan; 5) Division of Psychiatry and Behavioral Proteomics, Osaka Univ Grad Sch Med, Suita, Japan.

Alzheimer disease (AD) is a complex multifactorial disease in which many genetic and environmental factors are involved. We have performed an association study using functional candidate gene approach. The Ethics Committee of Ehime University School of Medicine approved the study protocol. Patients were selected using NINCDS-ADRDA criteria for definite or probable AD. ADP-ribosyltransferase 3 (ART3) gene is significantly upregulated only in the AD hippocampus compared with control. We studied genotypes of the ART3 polymorphisms in 374 patients with sporadic AD and 379 healthy controls. Genotyping was performed using the TaqMan-PCR method. Among the three SNPs in the ART3 gene, one SNP showed different distribution between AD and control. The frequency of both alleles and genotypes was significantly increased in patients with AD than in control subjects (P 0.0001). In the APOE-4 non-carrier subgroup, the risk allele showed significant association (OR = 2.86; 95% CI = 1.84-4.43). These results indicate that the ART3 is a novel risk gene for AD regardless of the APOE genotype. ART3 has been reported to be associated with non-obstructive azoospermia (infertlity). However, there are no reports regarding the association of ART3 gene polymorphism with AD. Our data should be further examined by functional analysis of ART3 polymorphisms in AD.

A kinship-based modification of the Armitage trend test to address hidden population structure and small differential genotyping errors. *C. Rakovski, D. Stram* Preventive Med, Univ Southern California, Los Angeles, CA.

Aims: We propose a modification of the well-known Armitage trend test to address the problems associated with unaccounted population structure in genome-wide case-control studies. We also provide insights into the analysis of family data from the perspective of populations with multiple highly correlated strata. Methods: The new test adopts beneficial traits from three existing testing strategies: the Eigenstrat, mixed model, and genomic control while avoiding some of their disadvantageous characteristics, such as the tendency of the principal components method to over-correct in certain situations or the failure of the genomic control approach to reorder the adjusted tests based on their significance levels. The new procedure is based on Gauss-Markov estimators derived from a straightforward linear model with an imposed variance structure proportional to an empirical relatedness matrix. Conceptual and analytical similarities to and distinctions from other approaches are emphasized throughout. Results: Our simulations show that the power performance of the proposed test is extremely promising compared to all competing strategies. The power gains are especially large when small differential DNA differences between cases and controls are present. Lastly, the new test offers adequate power even when applied to family data with discordant sib pairs and missing parental genotypes. Conclusion: The proposed modified approach attains higher power than that of the existing commonly implemented tests. Its performance improvement is dramatic when systematic differences between cases and controls exist.

BRACHYTELEPHALANGIC CHONDRODYSPLASIA PUNCTATA. A RARE X-LINKED SKELETAL DYSPLASIA PRESENTING AS PIRIFORM APERTURE STENOSIS AND CAUSED BY MUTATION IN THE ARYLSULFATASE E (ARSE) GENE. *S. Miller[1], B. Chung[2], D. Chitayat[2]* 1) Diagnostic Imaging, Hospital for Sick Children, Toronto, Ontario, Canada; 2) Clinical & Metabolic Genetics, Hospital for Sick Children, Toronto, Ontario, Canada.

Brachytelephalangic chondrodysplasia punctata (B-CDP) is a relatively benign form of CDP. It was first described in 1976 & later discovered to be X-linked & caused by a defect in the arylsulfatase E (ARSE) gene. We report a patient with B-CDP presenting with respiratory distress & narrowed nasal passage. Case report: Our patient was born at 34 weeks. His mother had GDM requiring insulin. Otherwise the pregnancy was uncomplicated & antenatal ultrasounds were normal. There was no maternal history of autoimmune diseases or teratogen exposure. Delivery was spontaneous & vaginal. His growth parameters were all normal. He had depressed nasal bridge & other unique facial features, short fingers with broad finger tips & hypotonia. He had no cataract & ichthyosis. Recurrent respiratory distress required repeated oral intubations as there were difficulties in passing the tube nasally. CT scan showed right anterior piriform aperture stenosis. Additionally, the nasal cartilage was deficient & there were stippling at the paranasal sinuses, nasal septum, C1 and odontoid. Skeletal survey confirmed the puncta at C1 & revealed additional puncta at various areas. The distal phalanges of digits appeared shortened and broadened. The karyotype was 46,XY & maternal screening for lupus was negative. Analysis of the ARSE gene showed a hemizygous A>G substitution in exon 7, causing p.Thr306Ala. This has not been previously reported. The threonine codon is evolutionarily conserved & is probably disease-causing. Functional analysis is in progress. From a recent review, so far 57 male patients with features of B-CDP have undergone ARSE testing, mutations have been detected in 31 but there were no obvious genotype-phenotype correlation. Respiratory problem is present in 30% of cases. With the prominent nasal hypoplasia, obstructed nasal passage causing respiratory distress is expected to be common. High index of suspicion is important for making the diagnosis.

A Novel Generalized Hypertrichosis - Scoliosis - Spina Bifida Syndrome: Phenotypic Features and Genetic Mapping. *X. Zhang[1], D. D. Shang[1], H. W. Zhu[2], Q. Liu[1], M. Sun[1], Y. Ao[1], W. Yang[1], T. Jing[2]* 1) Department of Medical Genetics, Peking Union Medical College, Beijing, China; 2) Center for Genetic Medicine, Lanzhou University, Lanzhou, China.

X-linked congenital generalized hypertrichosis (CGH) was mapped to chromosome Xq26-q27 in 1994. However, the disease gene has not yet been identified. We found a large Han Chinese family with CGH. Affected males in the families have severe hypertrichosis, scoliosis and spina bifida while the affected females only show mild hypertrichosis, suggesting an X-linked inheritance. To the best of our knowledge, this is the first report of an X-linked genetic syndrome that has a combination of the three manifestations. We performed genotyping and two-point linkage analysis using microsatellite markers on Xq to define the disease locus. The critical region was reduced to a 5.6cM interval at Xq26.3-q27.2. Mutation screening was preformed in all of the annotated genes (25 known genes with 222 exons), the pseudogenes (25 genes with 41 exons), some of the highly conserved non-coding sequences (54), all of the annotated non-coding RNA sequences (3), and some predictive transcription factors binding sites (6). The results didn not reveal any disease-causing mutation. We also extracted mRNA from skin fibroblast cells and skin tissues both from patients and normal controls, and performed RT-PCR to examine several candidate genes. Comparison between the amplified fragments in both subjects revealed no significant differences. Using the Affymetrix Genome-Wide Human SNP Array 6.0, we did not detect copy number changes in the critical region. In summary, we report a new X-linked genetic syndrome of generalized hypertrichosis accompanied with congenital scoliosis and spina bifida. We mapped the genetic locus to an interval of 5.6cM at chromosome Xq26.3-q27.2. Extensive mutation screening has not found potential pathogenic alterations.

Artemis cleaves cruciform-forming palindromic DNA leading to recurrent translocation in humans. *H. Inagaki[1], T. Ohye[1], H. Kogo[1], T. Kato[1], M. Tong[1], M. Tsutsumi[1], B. S. Emanuel[2], H. Kurahashi[1]* 1) Div Molecular Genetics, ICMS, Fujita Health Univ, Toyoake, Aichi, Japan; 2) Div Human Genetics, the Childrens Hospital of Philadelphia, Philadelphia, PA.

The t(11;22)(q23;q11) is a recurrent constitutional translocation in humans. The breakpoints on both chromosomes have been located within characteristic sequences, palindromic AT-rich repeats (PATRRs). We propose that the PATRRs form cruciform structures, and induce double-strand-breaks that are illegitimately repaired through non-homologous end joining. Since the breakpoints are mainly located near the center of the PATRRs, it is possible that the tip of cruciform DNAs are cut by Artemis, a hairpin opening enzyme in V(D)J recombination. We established a plasmid-based model system for the PATRR-mediated translocation using cultured cell lines. When Artemis was knocked down by siRNA, translocation-induced GFP signals decreased. Further, overexpression of Artemis increased the GFP signals in a dose-dependent manner. Combined with the data that the cruciform-forming PATRR was first cleaved diagonally in this model system, the translocation is generated from two successive cleavage reactions: diagonal cleavage of the PATRR by an unknown structure-specific nuclease, and hairpin-opening at the newly formed DNA ends by Artemis. Since the actual breakpoint sequences of human t(11;22)s were quite similar to those of this model system, our results strongly suggest cleavage of cruciform DNA structures as a mechanism that leads to palindrome-mediated translocation in humans.

Evaluating the performance of Affymetrix SNP 6.0 platform in the Japanese population. *N. Nishida[1], A. Koike[2], Y. Ogasawara[1], Y. Ishibashi[1], Y. Uehara[1], K. Tokunaga[1]* 1) Dept Human Genetics, Univ Tokyo, Tokyo, Japan; 2) Central Research Laboratory, Hitachi, Ltd, Tokyo, Japan.

Together with technology developments on large-scale single nucleotide polymorphism (SNP) genotyping, genome-wide association studies (GWAS) with hundreds of thousands of SNPs allow the identification of candidate genetic loci for multifactorial diseases in different populations. However, genotyping errors caused by genotyping platforms or genotype calling algorithms may lead to inflation of false associations between markers and phenotypes. In addition, the number of SNPs available for GWAS in the Japanese population has been investigated using only 45 samples in the HapMap project, which could lead to an inaccurate estimation of the number of SNPs with low minor allele frequencies. We genotyped 200 Japanese samples in order to estimate the number of SNPs available for GWAS in the Japanese population and to examine the performance of the current SNP Array 6.0 platform and the genotype calling algorithm Birdseed. About 20% of the 909,622 SNP markers on the array were revealed to be monomorphic in the Japanese population. Consequently, 661,599 SNPs were available for GWAS in the Japanese population, after excluding the poorly behaving SNPs. The Birdseed algorithm accurately determined the genotype calls of each sample with a high overall call rate of over 99.5% and a high concordance rate of over 99.8% using more than 48 samples after removing low-quality samples by adjusting QC criteria. We perform genome-wide SNP typing with the current SNP Array 6.0 platform, and perform high-resolution mapping with the DigiTag2 assay after genome-wide search for disease susceptibility regions. We are currently carrying out GWAS for various multifactorial diseases as joint research projects in order to identify candidate susceptibility or resistance loci in the Japanese population.

A study of single nucleotide polymorphism at PCSK9 gene in Chinese elders with hypercholesterolemia. *M. M. GU[1,3], X. L. WU[1,3], X. F. Pang[2,3], H. M. Yang[1], H. D. Song[2], Z. G. Wang[1,2]* 1) Department of Medical Genetics, Shanghai Jiao Tong University School of Medicine, Shanghai, China; 2) Ruijin Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China; 3) These authors contributed equally to this work.

Familial hypercholesterolemia (FH) is characterized by raised serum LDL cholesterol levels leading to accelerated atherosclerosis and increased risk of premature coronary heart disease. FH is commonly caused by some mutations in the LDLR, APOB or PCSK9. To investigate single nucleotide polymorphism (SNP) of PCSK9 (Proprotein convertase subtilisin/kexin type 9) gene in hypercholesterolemia group of Chinese elders, We investigate 73 patients (average age 70.269.54) from Ruijin hospital. Sequences spanning the exons, intron-exon boundaries, 5 and 3UTR of PCSK9 gene were detected by PCR-sequencing. A total of 22 SNPs were identified for the PCSK9 gene. All of them were transition. Five novel SNPs were found in our results, including R93V and A168V with amino acid change. Two variants, I474V and E670G which are related with LDL-C according to the report before, also were detected in this group. The hypercholesterolemia group of Chinese elders has not only the reported SNP changes at I474V and E670G, but also changes at the new SNP site of R93C and A168V, which are discovered for the first time in PCSK9 gene. Results obtained in this study provide some suggestive information for association studies on the plasma level of LDL-C with the PCSK9 variants in the hypercholesterolemia group of Chinese elders.

Sperm mitochondrial ATPase gene mutation and low seminal antioxidant levels in idiopathic asthenozoospermic men. *R. Dada[1], R. Kumar[1], S. Venkatesh[1], M. Tanwar[1], MB. Shamsi[1], R. Kumar[2], NP. Gupta[2], A. Bhat[3], RN. Bamezai[3], RK. Sharma[4]* 1) Laboratory for Molecular Reproduction and Genetics, Department of Anatomy, All India Institute of Medical Sciences, New Delhi, India; 2) Department of Urology, AIIMS, New Delhi, India; 3) School of life sciences, JNU, New Delhi, India; 4) ART center, Army Research and Referral Hospital, New Delhi.

Approximately 15% of the married couples failed to achieve parenthood after one year of regular unprotected intercourse, where approximately 40% of the male factor infertility is diagnosed as idiopathic. Seminal antioxidant enzymes, which maintain the physiological levels of ROS are lowered in known and unknown pathological conditions. Since mitochondrial DNA (mtDNA)is the key genome for the production of energy in the form of ATP, they are also both source and target of ROS. The present study includes 40 infertile asthenozoospermic men and 30 proven fertile controls. Semen analysis (WHO,1999)and seminal Catalase, glutathione peroxidase, and malondialdehye (MDA) levels were estimated. mtDNA was amplified and sequenced by standard PCR-DNA sequencing protocol. Infertile men had significantly lowered sperm count (43.3 4.1 Vs 62.1 16.2) and percent progressive motility (11.8 7.0, Vs 66.3 10.4) than fertile controls. Semen MDA levels of infertile group was found to be significantly higher, where catalase, and glutathione peroxidase (GPx) levels were significantly lower in infertile men compared to control men. mtDNA sequencing showed high frequency of nucleotide changes in the ATPase 6 (8279, 8280, 9098 & 8394) and ATPase 8 (8701, 8860 & 8879) genes of infertile men as compared to controls. Hence increased lipid peroxidation and low antioxidant levels induced oxidative stress coupled with ATPase mutation in OXPHOS pathway may be involved in impaired motility in such cases. Thus chronic exposure of the sperm to the high levels of ROS and lipid peroxidation may lead to irreversible changes in the mitochondrial and nuclear DNA. Early diagnosis of low antioxidant levels and oxidative stress and prompt management with antioxidants may prevent irreversible DNA damage in such cases.

Association testing with principal-components-based correction for population stratification. *M. Abney[1], M. S. McPeek[2]* 1) Dept Human Genetics, Univ Chicago, Chicago, IL; 2) Dept of Statistics, University of Chicago, Chicago, IL.

We consider the use of principal components (PC) to correct for population structure in case-control association testing. We propose a new method, PC-POPCORN, which could be considered an improvement to the EIGENSTRAT method of Price et al. 2006. In a simple class of models that include population substructure and admixture, we demonstrate that PC-POPCORN gives a test statistic that is correctly calibrated under the null hypothesis of no association, in the sense that the test statistic can be shown to be asymptotically chi-square-distributed in the presence of population structure, while EIGENSTRAT does not. We demonstrate circumstances under which PC-POPCORN performs well, but EIGENSTRAT gives inflated type 1 error or reduced power, while in other cases, both methods perform equally well. More generally, we provide an explicit justification for the use of PC to correct for population structure in case-control association testing.

The influence of choline or riboflavin intake and mild methylenetetrahydrofolate reductase deficiency on reproductive outcomes and congenital heart defects in mice. *J. Chan, L. Deng, R. Rozen* Human Genetics, McGill University, Montreal, Quebec, Canada.

Dietary and genetic disruptions in folate metabolism may affect embryonic development due to the essential role of folate in methylation reactions and nucleotide synthesis. A common polymorphism in methylenetetrahydrofolate reductase (MTHFR), 677CT, is found in 10 - 15% of North American and European populations, and results in mild MTHFR deficiency in homozygous TT individuals. Previous studies by our group have shown that low dietary folate and mild MTHFR deficiency increase reproductive loss, intrauterine growth delay and congenital heart defects in mice. However, the relationship between MTHFR and other nutrients involved in folate metabolism, especially choline and riboflavin, remains to be understood. In this study, the effects of low dietary choline and low dietary riboflavin, with or without MTHFR deficiency, were examined in murine embryos and placentae. Female *Mthfr* +/+ and *Mthfr* +/- mice were fed either control diet (CD), choline-deficient diet (ChDD) or riboflavin-deficient diet (RbDD) prior to mating with male *Mthfr* +/- mice. Embryos were collected at day 14.5 and examined for gross abnormalities, reproductive outcomes and heart defects. A significant decrease in the number of viable embryos and a trend toward decreased number of eggs released was observed in ChDD mice when compared to CD groups. When RbDD mice were compared with those fed CD, a significant increase in delay rate was seen, which was reflected in significant decreases in both embryonic weight and crown-rump length. Ventricular septal defects (VSDs) were observed in embryos of all 6 groups, with a general trend toward increased VSD incidence in embryos from ChDD and RbDD groups when compared to those of the CD. A decrease due to *Mthfr* genotype within the CD group was observed for both right and left ventricular compact wall thickness, with the latter reaching significance. Additional litters are being examined, but these preliminary data suggest that disturbances in folate, choline or riboflavin metabolism may influence reproductive outcomes and cardiac development.

Adult Phenotype of Mulvihill-Smith Syndrome: Early-onset tumor and sleep disorder. *K. Kosaki[1], T. Yagihashi[1], M. Kato[2], K. Izumi[1,3], R. Kosaki[1,4], T. Takahashi[1]* 1) Dept Pediatrics, Keio Univ, Tokyo, Japan; 2) Dept Neuropsychiatry, Keio Univ, Tokyo, Japan; 3) Dept Genet, Case Western Reserve University, Cleveland, OH; 4) Dept Clin Genet & Mol Med, Natl Ctr for Child Health & Dev, Tokyo, Japan;.

Mulvihill-Smith syndrome (MSS) is characterized by premature aging, multiple pigmented nevi, lack of facial subcutaneous fat, microcephaly, short stature, mental retardation and recurrent infections, however the adult phenotype of MSS has yet to be delineated. We report a 28-year-old woman with Mulvihill-Smith syndrome, who suffered from a pancreatic tumor at age 17 years. Her distinctive sleep pattern includes severe insomnia with disappearance of sleep spindles and K-complexes, persisting muscle tone, and loss of slow wave sleep. The clinical and neurophysiological studies are compatible with Agrypnia excitata, which is ascribed to a dysfunction of the thalamo-limbic system. Brain MRI and SPECT studies revealed structural and functional deficits in the dorsomedial region of thalamus and indicated that the alteration of thalamo-limbic system may underlie the sleep disturbances with MSS. Furthermore the rapid and severe decline in acquired cognitive function showed the distinct cognitive impairments like dementia including intellectual deficits, memory disorder and executive dysfunction. We posit that an early onset tumor, sleep disorder and cognitive decline are unique adult manifestations of Mulvihill-Smith syndrome.

Disease gene mapping of young-onset hypertension in the Taiwanese population. *H.-C. Yang[1], K.-M. Chiang[2], Y.-J. Liang[1], J.-W. Chen[3], Y.-T. Chen[2], W.-H. Pan[2]* 1) Inst. Statistical Science, Academia Sinica; 2) Inst. Biomedical Sciences, Academia Sinica; 3) National Yang-Ming University, Taipei, Taiwan.

[**Introduction**] Young-onset hypertension (YOH) has been demonstrated to have stronger genetic component than its older counterpart. [**Materials**] We collected information and biospecimen from 1039 YOH patients with age-of-onset < 51 in the Academia Sinica Multi-Centered Hypertension Genetic Study. A two-stage association scan was carried out. In the 1st stage (genomewide association study, GWAS), 175 YOH patients with normal BMI, triglyceride and HDL-C levels were selected, and 175 age and sex-matched normotensive controls were selected from the Taiwan Han Chinese Cell and Genome Bank. All samples were genotyped with Affymetrix Human Mapping 100K Set. In the 2nd stage (confirmatory association study), a total of 1024 patients and 1023 controls were genotyped for the SNPs identified in GWAS with Sequenom MassArray. [**Methods**] Genomewide single-point association tests (exact conditional logistic regression), multipoint association tests (haplotype association test and p-value combination test) and pairwise gene-gene interaction tests (odds ratio difference test and conditional logistic regression) were carried out. Multiplicity of testing was adjusted using false discovery rate. The identified SNPs were further examined using the identical statistical tests based on large samples. [**Results**] GWAS in the 1st stage identified nine single SNPs, seven triplets of contiguous SNPs, six triplets of haplotypes and the top 32 pairs of interactive SNPs. In total, 35 identified SNPs in known or hypothetical genes were further examined. Among them, 10 SNPs on chromosomes 2, 4, 6 and 20 obtained verification in the 2nd stage. Interestingly, four SNPs in a hypothetical gene of chromosome 2 and three SNPs in *FSTL5* of chromosome 4 were confirmed by multiple association tests. One SNP in *IMPG1* of chromosome 6 interacted with two SNPs on chromosome 20 and jointly contributed to YOH. *FSTL5* functions as a calcium-ion binder and *IMPG1* is particularly over-expressed in cardiac myocytes. Their roles on YOH were not reported by other authors and are being examined in our replication and functional studies.

Association of five candidate gene polymorphisms in Type 2 Diabetes and Diabetic Nephropathy. *Q. Hasan[1,2,4], S. Movva[2], S. Mubigonda[3], S. Saharia[3], Y. R. Ahuja[4]* 1) Department of Genetics , Kamineni Hospitals, Hyderabad, Andhra Pradesh, India; 2) Department of Genetics, Bhagwan Mahavir Hospital & Research Centre, A.C.Guards, Hyderabad; 3) Renal Unit, Bhagwan Mahavir Hospital & Research Centre, A.C.Guards, Hyderabad; 4) Department of Genetics, Vasavi Hospital & Research Centre, Lakdi-ka-pool, Hyderabad.

Type 2 Diabetes Mellitus (DM) is a chronic metabolic disorder that has a significant impact on the health, quality of life and life expectancy. Renal failure is a common and serious complication of longstanding type 2 DM. Asian Indians, an ethnically distinct population, lead the world in the number of people with type 2 DM and have a consequent epidemic of diabetes associated micro- and macro-vascular complications. There is no simple genetic model adequately explaining risk for diabetes and its complications, there are likely to be multiple genes with small to modest effects that interact with each other and with the environmental factors to affect susceptibility. A total of 336 individuals including 236 Type 2 DM (136 with and 100 without Nephropathy) and 100 healthy volunteers were assessed for polymorphism in five candidate genes: ACE (I/D), MTHFR (rs1801133), eNOS (exon7, NM_000603.3), MMP9 (rs3918240) and IGF2 (exon7, NM_000612.3). Individually MTHFR (p= 0.0003, OR= 4.0423), eNOS (P= 0.0073, OR= 7.3218) and MMP9 (P= 0.0111, OR= 2.1801) gene polymorphisms were associated with type 2 DM while ACE (p=0.027, OR=1.87) and IGF2 ($p<0.05$) were significantly linked to DN. Results of Multiple regression analysis indicated an interaction of ACE, MMP9 and IGF2 (t-value of 3.2374, 3.24414 and 2.79313 with a $p<0.05$) genes in increasing the risk of diabetic patients to develop DN. Individuals with impaired glucose tolerance, a positive family history of DM or other risk factors should be screened for MTHFR C677T, eNOS T1187G, and MMP9 C1562T as biomarkers while individuals with type 2 DM should be assessed for ACE D allele and IGF2 857 del A to identify diabetic patients with higher chance of developing DN. This will help in devising appropriate presentation and management strategies.

Severity Transmission Test: An approach to detect modifier genes affecting severity of disease. *z. zaheer[1,2], M. Devoto[2]* 1) Dept. of Statistics, Univ. of Peshawar, Pakistan; 2) The Childrens Hospital of Philadelphia, Univ. of Pennsylvania, Philadelphia, PA.

Many Mendelian disorders are characterized by an enormous variability in the expressivity of the phenotype in different individuals even in the presence of the same mutation at the major disease-causing gene, or by reduced penetrance. In the case of autosomal dominant disease, parents of severely affected children sometime are identified as asymptomatic carriers of a disease mutation only after the child is diagnosed. One explanation for reduced penetrance or variable expressivity of a disease phenotype is the presence of modifier genes. When one parent has a dominant mutation but is mildly or not affected and a child with the same mutation is severely affected, a possible interpretation is that the mildly affected or unaffected carrier parent and the severely affected child carry a different allele at a modifier gene. If this is true, then the modifier gene allele causing a more severe disease phenotype in the affected child must have been inherited from the non-carrier parent. Based on this hypothesis, we developed the Severity Transmission Test (STT), which can be used for the detection of modifier genes when unaffected carrier parent/affected child pairs are available. The test is designed to distinguish between the transmission from the carrier and non-carrier parents to the affected child.Under the assumption that the modifier gene allele in the affected child must be transmitted from the non-carrier parent, we define our statistic as a test of the null hypothesis that the number of times an allele is transmitted by the non-carrier parents is the same as the number of times that allele is not transmitted by the carrier parents. We conducted a simulation to evaluate the type I error and calculate the power of STT for different disease models. The power of STT was also compared to that of the Transmission Disequilibrium Test (TDT). We show that the STT maintains the nominal level of significance and is as powerful as the TDT, with the additional advantage that it can be used in the absence of the non-carrier parents, when only DNA from the carrier parents is available.

Alu-related 5q35 microdeletions in Sotos syndrome. *N. Matsumoto[1], J. Mochizuki[1,2]* 1) Dept Hum Genet, Yokohama City Univ Grad Sch Med, Yokohama, Japan; 2) Dept Obtet Gynecol, Kitasato Univ Sch Med, Sagamihara, Japan.

Haploinsufficiency of the NSD1 gene due to 5q35 microdeletions or intragenic mutations causes Sotos syndrome (SoS). In 46 of the 49 Japanese deletion-cases, common deletion breakpoints were located at two flanking low copy repeats (LCRs), implying that non-allelic homologous recombination (NAHR) between LCRs is the major mechanism for the common deletion. In the other three cases of atypical deletions, the mechanism(s) of deletions remains unanswered. We characterized the atypical microdeletions using fluorescence in situ hybridization (FISH), quantitative real-time PCR (qPCR), and Southern blot hybridization. All the deletion breakpoints in the three cases were successfully determined at the nucleotide level. Two deletions are 1.07 Mb (SoS19) and 1.23 Mb (SoS109) in size, and another consisted of two deletions with sizes of 28 kb and 0.72 Mb, intervened by an intact 29-kb segment (SoS44). All deletions were smaller than a typical 1.9-Mb common deletion. Alu elements were identified in both deletion breakpoints in SoS19, one of deletion breakpoints in SoS109, and both deletion breakpoints of the proximal 28-kb deletion in SoS44. Alu-mediated NAHR is strongly suggested at least in two of atypical deletions. Finally, qPCR is a very useful method to determine deletion breakpoints even in repeat-related regions.

Systematic Identification of Functional Networks in Human Heart Development. *L. A. Larsen[1,3], K. Lage[2], K. Møllgård[3], C. T. Workman[2], E. Bendsen[4], N. Tommerup[1,3], S. Brunak[2]* 1) Wilhelm Johannsen Centre for Functional Genome Research, Univ. of Copenhagen, Copenhagen, Denmark; 2) Center for Biological Sequence Analysis, Technical University of Denmark, Lyngby, Denmark; 3) Department of Cellular and Molecular Medicine, Univ. of Copenhagen, Copenhagen, Denmark; 4) Department of Obstetrics and Gynaecology, Univ. Hospital of Odense, Odense, Denmark.

Development of the human heart is tightly controlled in time and space by coordination of several molecular networks. Central players in cardiac development (CD) have been identified but the nature of molecular network and the degree of communication between the networks remains largely unknown. An experimentally derived network of 8855 human proteins generated by integrating data from thousands of protein-protein interaction (PPI) screens reported in the literature was used to identify functional network modules in CD and investigate how these modules interact.The functional modules were identified using a phenotypic ranking of protein complexes. We investigated the PPIs of 259 proteins known to be involved in CD from targeted mutations in mice. These 259 proteins can be assigned to 20 different morphological subcategories based on the cardiac phenotype of each mutation. The data showed that the CD proteins were significantly interconnected compared to random sets of proteins (P= 6.5e-07). We have identified a set of 49 novel CD candidates significantly interacting with proteins from the input set (adjusted p-values <0.051). The CD candidate genes were more differentially expressed during heart development compared to controls (P<0.006) and expression of selected candidates in tissue sections from human embryonic hearts correlate with their deduced function. Functional assignment of PPI subclusters within the 20 morphological subcategories supports that the heart is developed in a modular fashion. Furthermore our data show that there is extensive communication between different signalling pathways during heart development, and indicate a high degree of recycling of specific functional modules in a combinatorial fashion during the different stages of organogenesis.

Candidate of biomarkers for Fabry disease mouse model. *J. O. Choi, E. S. Park, J. S. Hong, H. Y. Park, S. C. Jung*
Department of Biochemistry, School of Medicine Ewha Womans University, Seoul, Korea.

Fabry disease is the lysosomal storage disease deficient for the alpha-galactosidase A, leading to alter glycosphingolipid metabolism and accumulate Gb3. Intravenous infusion of alpha-galactosidase A is used for curing patient with Fabry disease as enzyme replacement therapy (ERT). Gene expression in liver and kidney of male alpha-galactosidase A deficient mice of age were compared with that of wild type mice. Microarray analyses were performed before and after injections of alpha-galactosidase A. The identified genes were validated using quantitative real-time PCR and Western blot assay. Serum Amyloid A1(Saa1), S100 Calcium-binding protein A8 (S100a8), S100 Calcium-binding protein A9 (S100a9) and Lipocalin 2 (Lcn2) in liver and Neuropeptide Y (Npy) in kidney were significantly up-regulated compared with wild mice. The gene- expression was normalized after enzyme replacement therapy. Saa1 can be induced in mice with inflammatory stimulus. Lcn2 protein plays a role as a modulator of inflammation. This finding have been shown to modulate inflammation by saa1, S100a8, S100a9 and Lcn2. Npy also have been shown to regulate renal function. These genes could play an important role in pathogenesis of Fabry disease and provide promising targets for develop biomarkers to monitor disease progression and therapeutic efficacy in patients with Fabry disease.

Single nucleotide variations upstream of CHRNB3 affect reporter gene expression. *N. Hoft[1], J. Stitzel[1,2], J. Miyamoto[1], M. Ehringer[1,2]* 1) Institute for Behavioral Genetics, University of Colorado, Boulder, CO; 2) Department of Integrative Physiology, University of Colorado, Boulder, CO.

Recently single nucleotide polymorphisms (SNPs) in the upstream region of CHRNB3 have been associated with nicotine dependence (Bierut et al 2007, Saccone et al 2007) and early subjective effects of smoking (Zeiger et al 2008). The aim of this work is to assess whether these SNPs have a functional effect on gene expression. Promoter activity of fragments 3kb and 1.5kb immediately upstream of the CHRNB3 start codon has been characterized by cloning this region upstream of the luciferase gene in the promoterless reporter vector pGL3basic. Two constructs for each fragment length, representing both the major and minor haplotypes were compared. Promoter activity was tested in three cell types HEK293T, SH-SY5Y, and P19S18O1A1. Only P19S18O1A1 cells transfected with the 3kb constructs showed expression different than background. Lack of expression in the other two cell types suggests these may lack the transcriptional machinery necessary to express CHRNB3. Preliminary data indicate that the haplotype of minor alleles show an almost two fold increase (1.87 +/- 0.40) in promoter activity compared to the common haplotype. This difference indicates SNPs in this region may lead to functional differences in mRNA expression. Current efforts are now focused on examining individual SNPs on different haplotype background to characterize which SNPs in this region primarily account for this change in expression.

Identifying and Visualizing Complex Tumor Alterations through Integration of Diverse Data from The Cancer Genome Atlas (TCGA) Project. *J. Zhang[1], R. Finney[1], M. Edmonson[2], C. Schaefer[1], W. Rowe[1], C. Yan[1], R. Clifford[2], S. Greenblum[1], G. Wu[1], H. Zhang[2], H. Liu[2], C. Nguyen[2], K. Buetow[1,2]* 1) Center for Bioinformatics, NCI, Rockville, MD; 2) Laboratory of Population Genetics, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892.

Cancer is a complex disease driven by networks of altered genes. The Cancer Genome Atlas (TCGA) project is a large-scale comprehensive investigation into various mechanisms that lead to gene dysfunction in tumors. The first cancer studied by the TCGA project is glioblastoma multiforme (GBM). Genome-wide assays of somatic sequence mutations, copy number changes, gene and microRNA expression, and methylation data of GBM have been generated using multiple experimental platforms (http://tcga-data.nci.nih.gov/tcga/findArchives.htm). To facilitate the identification and visualization of the complex mechanisms that can lead to gene dysfunction, we have developed a web-based application, The Cancer Genome WorkBench (CGWB, http://cgwb.nci.nih.gov), to display the diverse, multi-dimensional TCGA data in an integrated form. CGWB presents a high-level summary view of the recurrently altered genes/regions in tumor and a high-resolution, detailed, expert view for analysis of the relationships between the various genomic data sets. A user can toggle between an interactive heat map view for gene, genome and pathway-orientated display of somatic sequence mutations, copy number changes, gene expression and methylation status data, and a genome-browser view which provides the association between the reference genome annotation with tumor aberrations. The workbench enables a user to evaluate correlation between somatic genetic alterations and gene expression. More importantly, it permits researchers to discover the complex molecular mechanisms underlying cancer from large volumes of diverse, multiple-platform, high-resolution data.

Requirement of Bardet-Biedl Syndrome Proteins for Leptin Receptor Signaling. *S. Seo[1,2], D.-F. Guo[3], K. Bugge[1,2], D. A. Morgan[3], K. Rahmouni[3], V. C. Sheffield[1,2]* 1) Dept. of Pediatrics, Univ. of Iowa, Iowa City, IA; 2) Howard Hughes Medical Institute; 3) Dept. of Internal Medicine, Univ. of Iowa, Iowa City, IA.

Obesity is a major public health problem in most developed countries and a major risk factor for hypertension and diabetes. Emerging evidence indicates that ciliary dysfunction contributes to human obesity but the underlying molecular and cellular mechanisms are unknown. Bardet-Biedl syndrome (BBS) is a genetically heterogeneous human obesity syndrome. BBS proteins are thought to play a role in cilia function and intracellular trafficking. Here, we show that BBS proteins are required for leptin receptor (LepR) signaling in the hypothalamus. Physiological data indicate that *Bbs2*, *Bbs4*, and *Bbs6* null mice are resistant to exogenous leptin even when serum leptin levels are normalized. In contrast, downstream melanocortin receptor signaling is unaffected, suggesting that obesity in BBS animals is due to defect(s) downstream or at the level of LepR but upstream of melanocortin receptors in the hypothalamic leptin-melanocortin axis. Reduced STAT3 phosphorylation upon leptin administration indicates that LepR signaling is attenuated in *Bbs2*, *Bbs4*, and *Bbs6* null mice. Impaired LepR signaling in BBS mice results in decreased *POMC* expression, which consequently leads to hyperphagia. Furthermore, we found that BBS1 protein physically interacts with the LepR and that loss of BBS proteins alters LepR trafficking. Our data indicate that BBS proteins mediate LepR trafficking and that impaired LepR signaling underlies energy imbalance in BBS. These findings represent a novel mechanism for leptin resistance and obesity.

Role of non-B DNA conformations in initiating the non-allelic homologous recombination-derived *Se^{fus}* allele and the interlocus gene conversion-derived *Sec1-FUT2-Sec1* hybrid allele. *J. M. Chen, C. Férec* Etablissement Français du Sang (EFS) - Bretagne; INSERM, U613; Université de Bretagne Occidentale (UBO), Brest, France. Jian-Min.Chen@univ-brest.fr.

A *Sec1-FUT2-Sec1* hybrid allele that apparently resulted from a gene conversion event has recently been reported. This allele is more appropriately termed *Sec1-Se^{428}-Sec1* because it is the *Se^{428}* mutant allele of the *FUT2* gene that acts as the donor sequence. Interestingly, the 5 half of the maximal converted tract (MaxCT) of this interlocus gene conversion event overlaps with the crossover region of the previously reported *Se^{fus}* mutant allele. The *Se^{fus}* allele was generated from non-allelic homologous recombination (NAHR), through which the 3-part of the *FUT2* gene was fused to the 5-part of the *Sec1* gene. Homologous recombination, including NAHR and gene conversion, is generally thought to be initiated by double strand breaks (DSBs). Recent studies have demonstrated that non-B DNA conformations (e.g. triplexes, hairpins and tetraplexes), rather than the DNA sequence *per se* in the orthodox right-handed Watson-Crick B-form, serve as recognition signals to induce chromosomal DSBs. That the MaxCT of *Sec1-Se^{428}-Sec1* overlaps with the crossover region of *Se^{fus}* may not be a mere coincidence; rather, it suggests that the initiating DSBs might have occurred within the overlapping sequence tract. Were this to be the case, the overlapping sequence tract should be capable of adopting non-B conformation(s). Indeed, four GGG repeats within the overlapping sequence tract may fold into a tetraplex structure. In addition, we identified a hairpin structure that can be formed by a pair of imperfect inverted repeats, by means of the previously established method for predicting local secondary structure of nucleotide sequences. These non-B DNA structures may act individually or synergistically to promote the formation of DSBs, which in turn initiated the process of homologous recombination. In summary, our findings provide a reasonable explanation for the occurrence of two different homologous recombination events within a short sequence tract.

Development of zebrafish model for Diamond-Blackfan anemia by antisense oligo-mediated knockdown of ribosomal protein S19 gene. *N. Kenmochi, T. Uechi, Y. Nakajima, A. Chakraborty, H. Torihara, S. Higa* Frontier Sci Research Ctr, Univ Miyazaki, Miyazaki, Japan.

Although the ribosome is essential for cell growth and development, the effects of ribosomal mutations and their role in human diseases has largely been ignored. Most people might think that defects in ribosomal components would cause serious problems with the translational apparatus and result in early embryonic death. However, recent study has revealed that the gene encoding ribosomal protein S19 (*RPS19*) is mutated in 25% of patients with Diamond-Blackfan anemia. Moreover, in mouse, Tail-short (*Ts*) and Belly spots and tail (*bst*) mutants have been identified as genetic abnormalities within *Rpl38* and *Rpl24* genes, respectively.

In this study, we developed an RPS19-deficient zebrafish by knocking down *rps19* using a Morpholino antisense oligo. The RPS19-deficient animals showed a dramatic decrease in blood cells as well as deformities in the head and tail regions at early developmental stages. These phenotypes were rescued by injection of zebrafish r*ps19* mRNA, but not by injection of *rps19* mRNAs with mutations that have been identified in DBA patients. Our results indicate that *rps19* is essential for hematopoietic differentiation during early embryogenesis. The effects were specific to *rps19*, but knocking down the genes for three other ribosomal proteins, *rpl35*, *rpl35a*, and *rplp2*, produced similar phenotypes, suggesting that these genes might have a common function in zebrafish erythropoiesis. The RPS19-deficient zebrafish will provide a valuable tool for investigating the molecular mechanisms of DBA development in humans.

SNPs at the Intelectin 1 gene confer risks for metabolic syndrome in a southern Taiwan population. *Y. Li[1], Y. Liao[1], M. Yu[4], S. Juo[1,2,3]* 1) Graduate Institute of Medical Genetics, Kaohsiung Medical University, Kaohsiung, Taiwan, Taiwan; 2) Department of Neurology, Kaohsiung Medical University, Kaohsiung, Taiwan, Taiwan; 3) Center of Excellence for Environmental Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan; 4) Hepatobiliary Division, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan.

Background and Purpose Dysregulation of the production of adipokines plays a pivotal role in the development of metabolic syndrome and cardiovascular comorbidity. Omentin, is able to enhance insulin-stimulated glucose uptake in adipocytes and consequently leads to obesity and insulin resistance. The purpose of present study is to investigate whether the polymorphisms at the gene encoding omentin (ITLN1) confer risks for metabolic syndrome in a southern Taiwan population.

Methods The diagnosis of metabolic syndrome is based on the modified version of NCEP ATPIII. We conducted a case-control association study using subjects with at least three of the five components of metabolic syndrome as cases and those with zero or one feature as controls. Five tagging SNPs and one additional non-synonymous SNP at ITLN1 gene were genotyped.

Results A total of 159 cases and 414 controls were included in the present study. Men were accounted for 47.8 % and the average age was 48.512.9 year-old. One SNP (rs952804) showed a borderline significance for metabolic syndrome. Compared to the common homozygotes CC (n=230), the rare allele carriers (CT: n=223 and TT: n=94) were associated with increased risks of metabolic syndrome (OR=1.50 and 1.38, p=0.057 and 0.245 respectively). We further evaluated the genetic effect on each of the five components of metabolic syndrome. SNP rs952804 appeared to have a dominant effect and was associated with elevated levels of fasting plasma glucose (p=0.010). We did not find any significant association between metabolic syndrome and the other five SNPs.

Conclusions The present study demonstrated that polymorphisms at the ITLN1 gene may influence fasting plasma glucose and predispose to the development of metabolic syndrome.

Makes-it sense to propose *CFTR* and *SPINK1* gene testing in patient with recurrent pancreatitis? A Belgian 8 years experience! *X. Pepermans[1], P. Deprez[2], A. Bosmans[1], K. Dahan[1]* 1) Center of Human Genetics, Cliniques Universitaires St-Luc, Bruxelles, Belgium; 2) Department of Gastroenterology, Cliniques Universitaires Saint-Luc, Bruxelles, Belgium.

Hereditary Pancreatitis (HP) is an autosomal dominant disease typically consisting of recurrent episodes of acute pancreatitis, progression to chronic pancreatitis and an increased risk of pancreatic cancer. Mutations in the cationic trypsinogen gene (*PRSS1*) cause the disease in 60% of the kindred while the roles of other genes e.g. *SPINK1* and *CFTR* are still under evaluation, especially in determining chronic pancreatitis: chronic pancreatitis or acute recurrent pancreatitis is considered to be a complex multigenic disease. Here, we have performed genetic tests for recurrent pancreatitis in 331 probands referred to our center from 1999 until 2007. Whole coding region of *PRSS1* was analyzed while a specific exon 3 scanning for *SPINK1* and screening for a panel of 36 common *CFTR* causing mutations were done. Seventeen of 331 patients (5,1%) had a disease causing mutations in *PRSS1*. Another 9,8% (15/152) of the patients had a *SPINK1* gene substitutions -heterozygote p.N34S in 9/152 and homozygote p.N34S in 1/152 -. Thirteen-seven of 331 (11%) had mutations in *CFTR* by mutation targeted testing. The futher complete *CFTR* screening (sequencing and MLPA) revealed that 3 of them were compound heterozygote whereas, 16 heterozygotes (16/331) were carriers of a common *CFTR* haplotype c.869+11C>T(rs1800503)-c.1393-61A>G(rs34855237)-p.Met470Val(rs213950). Of 37 patients with *CFTR* mutations 1% (3/331) also carried a *PRSS1* mutations and 2% (3/152) carried a *SPINK1* mutation moreover one individual was trans-heterozygote *PRSS1/SPINK1*. In total, multi-step testing of *PRSS1*, *SPINK1* an *CFTR* genes identified genetic variants in 62 Belgian patients considered by there physicians as candidate for genetic testing. These data fully illustrate the importance of consensus guidelines for indications, regarding our apparent low rate of mutation detection.

CD40 ligand gene polymorphisms in Taiwanese women with cervical squamous cell carcinoma. *T. Y. Chang[1], Y. C. Yang[1,2,4], Y. J. Lee[1,3,5], T. H. Su[2,4], W. F. Chen[1], H. W. Chan[1], H. F. Liu[1], M. Lin[1]* 1) Medical Research Dept, Mackay Memorial Hosp, Taipei, Taiwan; 2) Dept of Gynecology and Obstetrics, Mackay Memorial Hosp, Taipei, Taiwan; 3) Dept of Pediatrics, Mackay Memorial Hosp, Taipei, Taiwan; 4) Mackay Medicine, Nursing and Management College, Taipei, Taiwan; 5) College of Medicine, Taipei Medical University, Taipei, Taiwan.

Human papillomavirus (HPV) is considered to be a necessary but not sufficient cause for cervical cancer. It is now recognized that host immunogenetic background play an important role in the outcome of HPV infection and the subsequent development of cervical cancer. CD40 ligand (CD40L, also known as CD154 or gp39), a type II transmembrane protein, is expressed primarily on activated T cells and is indispensable to full and productive T cell activation. The aim of this study is to investigate if polymorphisms of the *CD40L* gene are associated with HPV-induced cervical cancer in the Taiwanese population. The *-3459 A/G* and *IVS4+121 A/G* polymorphisms were genotyped in 138 cervical squamous cell carcinoma (CSCC) patients and 519 age/sex matched healthy controls by using the Pre-Developed TaqMan Allelic Discrimination Assay. The presence and genotypes of HPV in CSCC patients were determined by PCR. We found no significant association between any polymorphisms or haplotypes examined and overall CSCC risk. In addition, no significant association was observed between HPV-16 positive CSCC patients and controls. Our findings provide no support for the hypothesis that *CD40L* polymorphisms are associated with increased risk for CSCC in the Taiwanese population.

New correction algorithms for multiple comparisons in case-control multilocus association studies based on haplotypes and diplotype configurations. *K. Misawa[1], S. Fujii[2,3], T. Yamazaki[2], A. Takahashi[2], J. Takasaki[2], M. Yanagisawa[3], Y. Ohnishi[2], Y. Nakamura[2], N. Kamatani[2,4]* 1) Research Program for Computational Science, RIKEN, Minato-ku, Tokyo, Japan; 2) SNP Research Center, RIKEN, Minato-ku, Tokyo, Japan; 3) Department of Computer Science, Waseda University, Tokyo; 4) Department of Advanced Biomedical Engineering and Science, and Institute of Rheumatology, Tokyo Womens Medical University, Tokyo.

The multiple comparison problem arises in population-based studies when the association between phenotypes and multilocus genotypes is examined. Although Bonferronis correction is often used to cope with such a problem, it may yield too conservative conclusions because all of the tests are assumed to be independent. We have developed new correction algorithms for the test of independence between phenotypes and multilocus genotypes at loci in linkage disequilibrium. In one of the algorithms, the exact type I error rate is calculated for the independency test. We found that such exact probabilities can be calculated using a 128 CPU PC cluster if the numbers of cases and controls are not more than 50. As an alternative method, we developed algorithms to calculate asymptotically the type I error rates using a Markov-chain Monte Carlo (MCMC) sampler which provided a good approximation to values calculated by the exact method. When the new algorithms were applied to both simulation and real data, the real overall type I error rates for the loci in linkage disequilibrium were from one-third to half as high as those obtained by Bonferronis correction. These algorithms are likely to be useful for multilocus association studies for data obtained by case-control and cohort studies.

Epigenetic variation in newborn twins. *M. Ollikainen[1], E. J. Joo[1], R. Andronikos[1], L. Gordon[2], R. Morley[1], R. Saffery[1], J. M. Craig[1]* 1) Developmental Epigenetics, Murdoch Childrens Research Institute, Melbourne, VIC, Australia; 2) Bioinformatics Department, Murdoch Childrens Researh Institute, Melbourne, VIC, Australia.

The phenotype of an individual is a result of a complex interplay between genome, epigenome and environment. The genotype of an individual is established at conception and only small changes occur later, whereas the epigenome is under constant environmental influence. Epimutations more common than genetic mutations and thereby contribute to phenotypic differences between individuals, whether genotypically different or identical (twins). To investigate the association between epigenetic variation and phenotypic discordance, we are examining the differences in DNA methylation in newborn twins. Our aim is to study the epigenetic profile in at least 20 newborn monozygotic twin pairs of at least 15% birth weight discordance. To establish a benchmark for the epigenetic variation between newborns we examined a dizygotic twin pair, sharing 50% of their genotype and most of their in utero environment. Methylated DNA Immunoprecipitation (MeDIP) was performed as described [Weber et al Nature Genetics 2005;37 853]. Validation of the MeDIP approach included locus-specific analysis of known methylated and unmethylated genes. We then compared signal intensities from the immunoprecipitated fraction to the input fraction following hybridisation to Affymetrix GeneChip Human Promoter Array. Hybridisation signals were analysed using the Model-based Analysis for Tiling arrays (MAT) algorithm. Promoter regions that were identified as methylated in two replicate experiments were divided into two categories: methylated in both twins and methylated in one twin only, and ranked according to MAT scores. We found several hundred highly methylated regions shared by both twins and a large number of regions methylated in one twin only. Current studies are aimed at examining epigenetic variation in monozygotic twins with a focus on methylation profile within birth weight discordant twin pairs. To our knowledge, this is the first study to assess epigenetic variation in newborn twins.

Association of Aphallia and Lung Agenesis. *M. Gerard-Blanluet[1], V. Lambert[2], S. Khung-Savatovsky[3], ML. Jaquemont[1], L. Perrin[1], C. Baumann[1], AL. Delezoide[3], A. Verloes[1]* 1) Medical Genetics, APHP Robert Debré Hospital, Paris, France; 2) Prenatal diagnosis, West Guyane Hospital Center, Saint Laurent du Maroni, Guyane, France; 3) Developmental Biology Department, APHP Robert Debré, Paris, France.

Aphallia, or congenital absence of the penis is a rare malformation, with an estimated frequency of 1/30 millions births. Aphallia is frequently associated with renal aplasia/dysplasia and imperforate anus. Absence of penis may be a part of severe malformation complexes that involve the perineal area, such as sirenomelia, cloacal extrophy or the anorectal septum malformation sequence. It has been associated with infants of diabetic mothers (Gripp et al., 1999). We report a patient with aphallia in a sequence of anorectal septum malformation associated with an unilateral lung agenesis. The fetus was the seventh child of non-consanguineous parents. Oligoanamnios was discovered at 32 weeks. Ultrasonography disclosed severe bilateral renal dysplasia with no residual renal function, and termination of the pregnancy was done at 35 weeks. No maternal diabetes was documented. Fetopathological examination showed the association of bilateral renal dysplasia, imperforate anus, aphallia with normal scrotal formation and testes, and right lung agenesis. Myocardic hypertrophy was present. X-rays showed thoracic hemivertebrae, and 14 ribs on the left side. Histological examination did not revealed any erectile tissue in pubic region. Karyotype was 46,XY normal. Aphallia and urogenital malformations are thought to be due to a defect of induction of the cloacal sequence during early embryogenesis. No lung agenesis are reported in the published cases of aphallia at our knowledge. Lung agenesis has been described in VACTERL association and oculo-auriculo-vertebral spectrum. The association of right lung agenesis, with presence of disorganization of the rib cage (hemivertebrae, 14 ribs on the left side), without maternal diabetes, integrate aphallia with imperforate anus and renal dysplasia in the larger group of blastogenesis disorders.

Three de novo deletions, one insertion, and one inversion of chromosome 6 in a patient with complete absence of expressive speech and reduced pain perception. *E. Passarge[3], M. Poot[1], R. van 't Slot[1], R. Leupert[2], V. Beyer[2], T. Haaf[2]* 1) Department of Medical Genetics, University Medical Centre Utrecht, Utrecht, Netherlands; 2) Institut für Humangenetik, Johannes Gutenberg-Universität Mainz, Mainz, Germany; 3) Institut für Humangenetik, Universitätsklinikum Essen, Essen, Germany.

A known de novo deletion 6q(q13-q14) within a de novo pericentric inversion 6(p11.2q15) associated with a distinctive phenotype was re-investigated. A much more complex chromosomal rearrangement consisting of three non-contiguous deletions was found instead of a single deletion as determined by high resolution G-band analysis reported previously (E. Passarge, Cytogenet. Cell Genet. 91: 192-198, 2000). We employed the Infinium HumanHap300 Genotyping BeadChip SNP array and BAC-based FISH. This revealed three adjacent, but non-contiguous de novo deletions and one insertion. We confirmed the deletion 6q14 and determined its size to be 11.9 Mb. This deletion affects 27 genes, some of which are involved in growth regulation, tissue modeling, and pain sensation, and may be related to the patient's phenotype. The most distal deletion (towards the centromere) of two newly discovered deletions of 360 kb in 6p12.3 contains four genes, RHAG, CRISP1, -2, 3, and PGK2. The proximal new deletion on 6p12.2-p12.1 is 1.15 Mb in size and involves five genes, PKHD1, IL17, MCM3, EFHC1, and TRAM2. By combining the SNP array and FISH data we were able to completely map and to reconstruct this highly complex rearrangement in a single chromosome of paternal origin. The main clinical features of this 31-year-old women are dysmorphic facial features consisting of a broad face, prominent glabella, broad nose, and hypertelorism, non-progressive deficit of motor control, a broad-based slow-motion-like gait, absence of speech development, inability to acquire and comprehend theoretical knowledge, and reduced sensitivity to pain. This study shows that a complex chromosomal rearrangement disclosed by high resolution cytogenetic methods may be even more complex when analyzed at the molecular level.

Association of ITGAM and BLK polymorphisms with systemic lupus erythematosus (SLE) in Hong Kong Chinese. *MH. ZHAO, WL. YANG, YL. LAU* Department of Paediatrics and Adolescent Medicine, University of Hong Kong, Queen Mary Hospital, Hong Kong.

Systemic Lupus erythematosus (SLE) is an autoimmune disease characterized by high-level autoantibody production and immune complex deposition. Both genetic and environmental factors contribute to the etiology of SLE. In order to identify susceptibility genes associated with SLE risks in Chinese population, we have studied several SNPs in ITGAM and BLK, two genes found to be associated with the disease in Caucasians but with unknown effect in other populations. SNP rs1143682 and SNP rs1143683 of ITGAM, as well as SNP rs13277113 and SNP rs2248932 in BLK, were genotyped in this study by either TaqMan or direct sequencing of PCR products. We found that the risk allele A of rs13277113 in BLK is associated with SLE in Chinese (P=6.37×10-5, OR=1.39), and it is a major allele in our population while a minor allele in the Caucasians. It is enriched (77%) in SLE patients compared to healthy controls (71%). However, no significant difference was seen in ITGAM polymorphisms in Hong Kong Chinese. But when we separate the whole population by gender, for males, SNP rs1143682 has a G allele frequency of 46% in patients comparing to 34% in controls (P=0.009, OR=1.62). The conclusion is that BLK, but not ITGAM, is associated with Systemic Lupus Erythematosus (SLE) in Hong Kong Chinese. Whereas ITGAM seems to be a risk factor only in male patients, this finding needs to be verified by larger and independent sample collections, as male patient only constitute 10% of all the affected in our population.

Genetic variation and haplotypes of the *UGT1A1, 1A6* and *1A7* polymorphisms in Sao Miguel population (Azores, Portugal). *M. J. Brilhante[1], P. R. Pacheco[1,2], F. Sigallat[1], H. Polena[1], R. Cabral[1,2], C. C. Branco[1,2], L. Mota-Vieira[1,2]* 1) Molec Genetics & Pathol Unit, Hosp Divino Espirito Santo PD, EPE, Ponta Delgada, Azores Islands, Portugal; 2) Instituto Gulbenkian de Ciência, Oeiras, Portugal.

Glucurodination reactions, catalysed by uridine-diphosphate glucuronosyltransferase (UGT) enzymes, are a detoxification process that transforms both endogenous and exogenous compounds into glucuronic acid, aiding their excretion. This study is a starting point for the evaluation of the impact of UGT genotypes and haplotypes in the individual susceptibility to chemical-induced diseases and responses to therapeutic drugs in Sao Miguel population (Azores). Here, we assess the genome of 469 individuals by determining the prevalence of the polymorphisms and haplotypes in *UGT1A1*, *UGT1A6* and *UGT1A7* and by calculating the extent of linkage disequilibrium (LD) in the genomic region encompassing these genes. Allelic analyses disclosed that *UGT1A1* presented the major frequency for *1 allele (70.7%), being *UGT1A*36* and *UGT1A*37* the two rare alleles. These last alleles, found only in African-ancestry individuals, confirm our previous results on Sao Miguel genetic background, an admixed population composed of European, Jews and Africans. For *UGT1A6*, the allele with major frequency was *1 (68.0%). In *UGT1A7* the three higher frequencies were observed for *1 (34.6%), *2 (28.1%) and *3 (35.5%). Concerning genotypic analyses, the frequencies detected for *UGT1A1*: *1*1 (50.5%) and *1*28 (39.7%); *UGT1A6*: *1*1 (47.5%) and *1*2 (36.2%); and *UGT1A7*: *1*3 (24.7%), *1*2 (19.6%) and *2*3 (19.2%). Haplotype analysis of the *UGT1A1*, *UGT1A6* and *UGT1A7* polymorphisms showed that three major haplotypes constituted 79.9% of the allelic variants in the cohort. We identified 21 haplotypes being *UGT1A1*28-UGT1A6*2-UGT1A7*3*, the second most frequent (23.8%), in which all three alleles codify for low function UGT isoforms. These highly prevalent polymorphisms and haplotypes result in modified expression and activity of UGTs, may influence susceptibility to cancers, and predispose to side effects of drugs. Currently, we are estimating the linkage disequilibrium between *UGT1A1*, *UGT1A6* and *UGT1A7*. (Azorean Government Funded).

The involvement of neuronal migration genes in developmental dyslexia. *J. A. Chapman, D. Harold, G. Hill, M. C. O'Donovan, J. Williams* Dept of Psychological Medicine, Cardiff University, Henry Wellcome Building, Heath Park, Cardiff, United Kingdom.

Neuronal migration is the process by which neurons in the brain are correctly positioned during development. It is achieved though the rearrangement of cytoskeletal components in response to extracellular cues, mediated by numerous intracellular signalling pathways. Through the use of RNA interference (RNAi), a number of genes that have shown an association with developmental dyslexia (DD) have also shown evidence for a role within neuronal migration. These include ROBO1, DYX1C1, DCDC2 and KIAA0319.

Other genes within DD linkage regions are plausible candidates for involvement in neuronal migration, and we aimed to investigate variants within these genes for an association with DD in our case/control sample. The most promising genes that have been identified so far are DCDC2b, (which shows homology to DCDC2), KIAA0319L (which shows homology to KIAA0319) and CDC42, within the replicated DD linkage region 1p34-36. A gene within the DD linkage region on chromosome 15q21 called Protogenin (PRTG) may also have a role within neuronal migration.

We genotyped tag SNPs for each of these 4 genes (71 SNPs in total) in our UK sample of 357 cases and 269 controls using the Sequenom platform. None of the variants show significant allelic, genotypic or haplotypic association with DD in our sample. However, these genes are not an exhaustive list of neuronal migration candidates, and impaired neuronal migration may still have a role to play within DD.

Paget's disease of bone in the Italian population: novel SQSTM1/p62 mutations and genotype-phenotype correlations. *F. Gianfrancesco[1], L. Gennari[2], P. Fusco[1], T. Esposito[1], D. Rendina[3], A. Ciccodicola[1], D. Merlotti[2], V. De Paola[2], G. De Filippo[4], G. Martini[2], P. Strazzullo[3], R. Nuti[2], G. Mossetti[3]* 1) Institute of Genetics and Biophysics, Italian National Research Council, Naples, Italy; 2) Internal Medicine, Endocrine-Metabolic Sciences and Biochemistry, University of Siena, Italy; 3) Clinical and Experimental Medicine, Federico II University of Naples, Italy; 4) Unit of Pediatric Endocrinology, Gaetano Rummo Hospital, Benevento, Italy.

Paget disease of bone (PDB) is a chronic disease of the skeleton that affects up to 2-3% of the population aged > 50 years. The PDB geographic distribution is not uniform, with a higher prevalence of the disease in in populations of British descent. We recently characterized an area of increased prevalence of PDB in the region of Campania, in Southern Italy. Patients from this region showed increased severity of disease with peculiar phenotypic characteristics and an increased number of familial cases. We examined the clinical characteristics, and the prevalence and type of SQSTM1 mutations in a large sample of 380 unrelated PDB subjects from several regions including 145 patients from Campania. Nine different mutations in SQSTM1 gene were observed in 35.2% and 11.3% of familial and sporadic PDB cases, respectively (equivalent to 15.6% of the overall cohort). Five of these mutations, were novel and have not been previously described. An higher prevalence of SQSTM1 mutations was observed in polyostotic than monostotic cases (7% vs 22%; $p<0.005$). In keeping with previous studies, the P392L was however the most common observed mutation in both sporadic and familial cases. Genotype-phenotype analysis confirmed an increased severity of disease and an earlier age of onset. Interestingly, in PDB subjects from Campania a different distribution and a significantly reduced prevalence of mutations was observed with respect to the other regions, despite an increase in disease severity and the higher prevalence of familial cases. This might imply the presence of mutations in different genes as well an increased persistence of a possible environmental trigger.

Towards the identification of a new gene for Andersons disease. *M. Varret[1], M. Sylvain[2], J.-P. Rabès[1,3], A. Grodet[4], A. Munnich[1], L. Ferkdadji[5], C. Boileau[1,3], L. Aggerbeck[2], J. Schmitz[6], M.-E. Samson-Bouma[1]* 1) INSERM U781, Hopital Necker, Université Paris Descartes, Paris; 2) CGM, CNRS, Avenue de la Terrasse, 91198 Gif-sur-Yvette; 3) Laboratoire de Biochimie et de Génétique Moléculaire, CHU Ambroise Paré (AP-HP & Université Versailles-Saint-Quentin-en-Yvelines), Boulogne; 4) Université Paris 7 Denis Diderot, Faculté de Médecine Xavier Bichat, Paris; 5) Service dAnatomie Pathologique, Hôpital R. Debré, Paris; 6) Departement de Pediatrie, Hopital Necker-Enfants Malades, Paris, France.

The study of naturally occurring mutations in hypocholesterolemic patients presenting with lipid malabsorption syndrome has been useful in identifying new target for lipid-lowering therapy. Andersons disease, is a very rare lipid malabsorption syndrome, usually diagnosed in early infancy, characterized by an inability to export dietary lipids as chylomicrons due to a recessive genetic defect in lipoprotein secretion. Recently, the molecular basis of this defect was show to be due to mutations in the SARA2 gene encoding the Sar1b protein. This protein is involved in the vesicular transport between the endoplasmic reticulum and the Golgi. We report here 4 patients from 2 families, clearly diagnosed as Andersons disease patients. They had low levels of cholesterol and of lipid soluble vitamins. No chylomicrons were secreted after a fat load, and alpha and betalipoproteins were 50% of normal. Endoscopy showed a typical white stippling-like hoar frosting covering the intestinal mucosal surface. Ultrastructural examination of intestinal biopsies showed an accumulation of free lipid and lipoprotein-like particles, reflecting the secretory defect. Direct sequencing of the 7 exons of the SARA 2 gene revealed no mutation. This result thus excludes, in these patients, SARA2 as the molecular basis of the lipoprotein secretory defect and suggests the existence of at least another gene. A genome scan, performed in the two families allowed us to identify four candidate locus that we are investigating at the moment. Proteins involved in the intracellular processing of chylomicron secretion would be good candidates.

Copy number variation and chronic pancreatitis. *C. Férec, E. Masson, C. Le Maréchal, J. M. Chen* INSERM, U613; Université de Bretagne Occidentale (UBO); Etablissement Français du Sang (EFS) - Bretagne; Centre Hospitalier Universitaire (CHU) Brest, Hôpital Morvan, Brest, France. claude.ferec@univ-brest.fr.

Although hereditary pancreatitis was described as an autosomal dominant disease in the early 1950s, only in the past decade has tremendous progress been made toward unraveling its molecular basis. In 1996, a gain-of-function missense mutation in the cationic trypsinogen gene (*PRSS1*) was identified by a candidate gene approach. Thereafter, search for chronic pancreatitis-associated genetic factors has been largely focused on one form of genetic variation viz. single-nucleotide substitutions. Recently, we have identified both duplication and triplication of a ~605-kb segment containing *PRSS1* and *PRSS2* (encoding anionic trypsinogen) on chromosome 7q34 in French Caucasians with hereditary or idiopathic chronic pancreatitis, by means of quantitative fluorescent multiplex PCR (Le Maréchal et al. Nat Genet 2006; Masson et al. Clin Gastroenterol Hepatol 2008). Thus, in the context of *PRSS1*, both qualitative missense mutations and quantitative copy number mutations independently contribute to the etiology of chronic pancreatitis. More recently, we have characterized an intriguing hybrid *PRSS2*/*PRSS1* gene, in which exons 1 and 2 are derived from *PRSS2* and exons 3 to 5 from *PRSS1* (Masson et al. Hum Genet 2008). Interestingly, this hybrid gene causes the disease through a combination of its inherent double gain-of-function effect, acting simultaneously as a quantitative copy number mutation and a qualitative missense mutation; this reveals a previously unknown mechanism causing human inherited disease, enriches the lexicon of human genetic variation and goes beyond the known interaction between copy number variations (CNVs) and single nucleotide substitutions in health and disease. It should also stimulate more interest in analyzing both types of genetic variation whenever one tries to determine the contribution of a specific locus to a given disease phenotype. In short, our findings made over the past two years represent a further demonstration of how studies of CNVs have altered the landscape of genetic research and offers a fresh glimpse into the exciting realm of human CNVs.

A Fourth Phenotype for Autosomal Dominant Hypercholesterolemia. *A. Marques[1], M. Marduel[1], J. Bonneau[1], M. Devillers[1], D. Erlich[1], K. Ouguerram[2], M. Krempf[2], M. Abifadel[1,3], J.-P. Rabès[1,4], C. Boileau[1,4], M. Varret[1]* 1) INSERM U781, Hosp Necker, Universtité Paris Descartes, Paris, France; 2) INSERM U539, University Hospital, Nantes, France; 3) Faculté de Pharmacie, Université Saint-Joseph, Beirut, Lebanon; 4) Laboratoire de Biochimie et de Génétique Moléculaire, CHU Ambroise Paré (AP-HP & Université Versailles-Saint-Quentin-en-Yvelines), Boulogne, France.

Autosomal Dominant Hypercholesterolemia (ADH) is due to defects in LDLR, APOB or PCSK9. Reports revealed the involvement of still unknown genes. We report an ADH family (HC6) excluding the 3 known genes and named the pathology HCHOLA4. We wish to identify HCHOAL4 and define the associated pathophysiology. A genome scan, performed in HC6 (11 affected), localized HCHOLA4 at 16q22.1. Functional candidate genes did not show any causal mutation. In vivo kinetics of apoB-100 showed a decrease in LDL catabolism. Q-PCR analysis of LDLR expression in EBV-transformed lymphoblasts showes that cells of 4 affected subjects do not reply to cholesterol deprivation by activating LDLR expression. These results suggest an alteration, direct or not, in LDL receptor endocytosis or intracellular traffic. We collected DNA from 25 small pedigree (63 affected) and excluded the 3 genes. Linkage to 16q is excluded in 8 families, the 17 other are being enlarged to allow reduction of the genetic interval. For 3 families, Q-PCR analysis of LDLR expression in lymphoblasts showed that 2 probands do not reply to cholesterol deprivation like affected subjects of HC6. One of these probands is from a familly (HC94) in wich linkage to 16q was excluded. A genome scan in HC94 (6 affected) with Illumina arrays began. We expect to redefine the HCHOLA4 interval with combined results from HC6 and HC94. Furthermore, 2-D electrophoresis for cytosolic proteins from lymphoblasts showed different profiles between affected and unaffected HC6 members. Mass spectrometry identified 120 proteins from the endocytosis, signaling or protein degradation pathway. These results will be confirmed by Q-PCR and westerns.

Effectiveness of enzyme replacement therapy in children: results from FOS - the Fabry Outcome Survey. *R. Hartung[1], C. Kampmann[1], R. Parini[2], G. Pintos-Morell[3], M. Beck[1], U. Ramaswami[4] on behalf of the FOS investigators*
1) University Children's Hospital, Mainz, Germany; 2) University Hospital San Gerardo, Monza, Italy; 3) University Hospital Germans Trias i Pujol, Badalona, Spain; 4) Addenbrookes University Teaching Hospital, Cambridge, UK.

Background: Fabry disease (FD), a progressive, X-linked lysosomal storage disease, leads to major organ failure and premature death. Enzyme replacement therapy (ERT) is now available but limited data exist on the long-term effectiveness of ERT in children. Methods: Effects of ERT with agalsidase alfa (Replagal; Shire HGT) were analysed at baseline, and after 12 and 24m of ERT in children aged 18y in FOS, a global database of patients with FD. Results: Of 257 children in FOS (120 boys, 137 girls), ERT data were available for: 49 boys, 26 girls at 12m; 31 boys, 21 girls at 24m. Diagnosis was delayed by 2y in both sexes. Onset of symptoms, diagnosis and treatment initiation occurred 2y later in girls than boys. Treatment for 24m reduced vomiting, fatigue, heat intolerance, abdominal pain, chronic pain and pain attacks. Renal function remained stable. Additional sign/symptom reductions included diarrhoea (boys), constipation, cold intolerance, tinnitus and microalbumin levels (girls). Results at 12m were similar. Cardiac structure was assessed by echocardiography in 12 boys and 9 girls (mean ages 13.2 and 14.8y, respectively) at baseline and 12m. Compared with a normal population of 2000 children, the mean z-score for left ventricular mass at baseline was 1.36 in girls (p0.01) and 0.73 (not significant; NS) in boys. After 12m of ERT the z-score was reduced in girls to 0.98 (NS) and was stable in boys (0.81; NS). Mean wall thickness z-scores decreased from 1.46 (p0.01 vs normal population) to 0.72 in girls (p0.05 vs baseline) and from 1.04 to 0.75 in boys (NS). Over 24m of ERT, 5 girls and 4 boys had reductions in cardiac dimensions (NS). Of the total cohort of treated children in FOS, 25 boys and 9 girls reported adverse events, the most common being mild, infusion-related reactions (n=50). Conclusions: ERT with agalsidase alfa for up to 24 months in children reduces or stabilizes the signs and symptoms of FD.

A new chromosomal translocation t(2q14;10p15.2) in a child with hypoparathyroidism. *L. TELVI[1], A. ROTHENBUHLER[2], J. GOGUSEV[3], M. MINZ[1], S. CHATAIGNIER[1], A. LINGLART[2], M. GARABEDIAN[2]* 1) Cytogenetics Laboratory, Hospital St Vincent de Paul, Paris, France; 2) Dept of Pediatric Endocrinology, Hospital St Vincent de Paul, Paris, France; 3) INSERM U567, Hospital Cochin, Paris, France.

Hypoparathyroidism (HPT) is an endocrine disorder in which hypocalcemia and hyperphosphatemia are the results of a deficiency of parathyroid hormone (PTH). We describe a case of idiopathic HPT in a 3 year-old girl showing a severe hypocalcemia (2.08 mmol/l) and a deficiency of the PTH (8 pg/ml). However, the clinical examination was normal including normal cardiac ultrasound either the immunological examinations with an IgA level at 1.04 g/l. During neonatal period, the patient had shown seizures and a low level of calcemia (1.4 mmol/l). The cytogenetics analysis using RHG banding showed a reciprocal translocation between chromosomes 2 and 10. FISH analysis confirmed the t(2q14;10p15.2) by using specific painting probes for chromosomes 2 and 10. We did not found deletion or duplication for the following genes : Tuple1(22q11),Shank3(22qter) and DGCR2(10p14). Otherwise, the DNA sequencing of calcium-sensing receptor gene (CASR) did not found any mutation. The diagnosis of idiopathic HPT was established and the patient was treated with calcitriol, which normalise the concentrations of calcemia and phosphatemia. The description of a new chromosomal translocation in a patient with HPT could lead to the location of susceptibility gene(s) involved in the pathogenesis of this disease. A candidate gene involved in the pathogenesis of HPT is underway by using array-CGH strategy and molecular analysis.

IN SILICO ANALYSIS OF PENDRIN. *E. Leonardi[1], S. Vanin[2], E. Orzan[3], A. Murgia[1], S. C. E. Tosatto[2]* 1) Department of Pediatrics, University of Padua, Padua, Italy; 2) Department of Biology University of Padua, Padua, Italy; 3) Pediatric Audiology Unit, Department of Otolaryngology and Otosurgery, University Hospital of Padua, Padua, Italy.

Mutations in SLC26A4 gene, which encode for pendrin, cause Pendred syndrome and hearing loss with enlarged vestibular acqueduct (EVA). The two disorders differ by the presence of thyroid defects. More than 150 SLC26A4 mutations have been reported but the genotype-phenotype correlation is not fully understood. We adopted an integrated approach for the study of pendrin combining available biological information, clinical data and bioinformatic results, to understand structure-function relationships and pathogenetic mechanisms. Family-based resources searches revealed that SLC26A4 contains three SLC26 family motifs: SLC26 sulfate transporter signature, sulfate transporter domain and STAS domain. Analysis of pendrin topology resulted in the prediction of 12 transmembrane domains. The program ZPRED predicts the second helix as a potential re-entrant region with a functional role in the anion selectivity. The identification of the GxxxG motifs in the last transmembrane helix, as a potential site of dimerization, provides structural support for the proposed model of dimeric quaternary structure of the SLC26 transporter. The 3D structure of the STAS domain, modeled with computational methods, was analyzed by structural comparison with similar folds to retrieve information on its possible role in the regulation of transport activity. A phylogenetic analysis was performed on the STAS domain sequence of the SLC26 transporter. We observed that the paralogs differ from each other by the length of the variable loop, which seems conserved in each member across species. This loop is suggested to function as an anion sensor and might be responsible for the conformational change in the STAS domain. The overall in silico characterization has lead to a structural model on which most mutations have been mapped. Together with a new hypothesis elucidating Pendrin structure-function relationships, we propose an explanation of the molecular effects of amino acid substitutions and their possible pathogenic role.

Clinical evaluation and molecular - genetic diagnosis of Wilson disease. *A. Krumina[1], J. Keiss[2], V. Sondore[2], G. Cernevska[3], A. Zarina[1], B. Lace[1]* 1) Dept Medical Biol & Genetics, Riga Stradins Univ, Riga, Latvia; 2) Infectology Center of Latvia, Riga, Latvia; 3) Children Clinical University Hospital, Riga, Latvia.

Wilson disease (WD) is an autosomal recessive disorder of copper metabolism caused by mutations in the gene *ATP7B*. As a consequence of enormous clinical variability of WD, the condition is commonly underdiagnosed. DNA analysis to identify defects in the *ATP7B* gene can provide unequivocal confirmation of WD in affected symptomatic or presymptomatic individuals. More than 300 mutations in the gene *ATP7B* causing WD have been reported. Among individuals of Northern European origin the most prevalent mutation is H1069Q. The data on correlation between WD patient genotypes and clinical presentation are controversial. Aims of the research were: 1) to perform mutation detection in seven exons of the gene *ATP7B* in the Latvian Wilsons disease patients; 2) to analyse the relationship between patients genotypes with mutation H1069Q and their clinical manifestation; 3) to analyse the predictive value of different clinical and biochemical data by comparing them with the results of DNA analysis. 40 patients with WD suggestive symptoms were included in the study. Laboratory confirmation of the clinical diagnosis included determination of serum copper, serum ceruloplasmin, liver function tests, 24-hour urinary copper excretion, and response to D-penicillamine. 157 unrelated healthy individuals were tested as controls for mutation H1069Q incidence analysis. Mutation H1069Q testing was performed by PCR Bi-PASA method. Seven exons of the gene *ATP7B* were sequenced. Five mutations (two previously described and three novel mutations) in the gene *ATP7B* were identified. The H1069Q mutation was present at 52.5% of the disease alleles. One individual among 157 healthy Latvians was found to be a mutation H1069Q carrier. Wide clinical variability was observed among individuals with the same *ATP7B* genotype. An algorithm for the diagnosis of WD, including testing for mutation H1069Q, is recommended for the populations where mutation H1069Q accounts for 50% of WD alleles or more.

An adult-onset autosomal dominant leukodystrophy linked to 5q23.2-q31.1 without duplication of the *LMNB1* gene. *A. Brussino[1], G. Vaula[2], C. Cagnoli[1], E. Panza[3], M. Seri[3], S. Leombruni[2], M. Bergui[2], G. B. Bradac[2], L. Pinessi[2], E. Di Gregorio[1], S. Scappaticci[4], S. Camanini[4], S. Cavalieri[1], E. Grosso[1], N. Migone[1], A. Brusco[1]* 1) Genetics Biology & Biochem, Univ Torino, Italy; 2) Neurosciences, A.O.U. S.Giovanni Battista, Torino; 3) Gynecological, Obstetric and Pediatric Sciences, Univ of Bologna, Italy; 4) Department of Biology and Medical Genetics, University of Pavia, Italy.

Adult-onset Autosomal Dominant LeukoDystrophy (ADLD, OMIM 169500) is a rare CNS demyelinating disease caused by duplication of the lamin B1 gene (*LMNB1*, 5q23). At present, five families, one recently identified by our group, are described with a homogenous phenotype. Other kindreds segregating an autosomal dominant form of leukodystrophy are reported, but their *LMNB1* gene status is at present unknown. We investigated the genetics of one such family in which affected members have a variant ADLD disease (vADLD). Patients become symptomatic in the 4th-5th decade, and in contrast with ADLD show mild involvement of the cerebellar white matter and no autonomic dysfunction at onset. An extensive search for *LMNB1*gene mutations was performed in this family using copy-number sensitive real-time PCR, coding region and cDNA direct sequencing, and Southern blot. No duplication/deletion and point-mutations were detected. A genome-wide scan identified two contiguous candidate regions in 5q23.2-q31.1: (1) between D5S2971-D5S2968 containing the Zinc finger 608 gene in which point mutations and genomic rearrangements were excluded by sequencing analysis and Southern blotting; and (2) between D5S804-D5S2115, a region comprising 67 transcripts, including *LMNB1*. Our preliminary results show the existence of a novel autosomal dominant adult-onset leukodystrophy linked to 5q23 and distinguishable from the ADLD phenotype both clinically (absence of dysautonomic initial symptoms) and genetically (absence of lamin B1 duplication). While our data tend to exclude *LMNB1* duplication as the cause of vADLD, we cannot rule out the possibility that *LMNB1* may harbor a mutation in an unknown regulatory region affecting mRNA expression or stability.

Quantitative MethylScreen DNA methylation analysis of the FXN gene. *S. Al-Mahdawi, R. Mouro Pinto, C. Sandi, M. A. Pook* Biosciences, Brunel University, Uxbridge, United Kingdom, School of Health and Social Care, Heinz Wolff building, Brunel University, Uxbridge, Middlesex, UB8 3PH, United Kingdom.

Friedreich ataxia (FRDA) is a neurodegenerative disorder caused by a homozygous GAA repeat expansion mutation within intron 1 of the FXN gene. The mutation causes reduced levels of the mitochondrial protein, frataxin, which is involved in iron-sulphur cluster and heme biosynthesis. Frataxin insufficiency leads to iron accumulation, oxidative stress and ultimately cell death. With the absence of effective treatment for this disorder, research in our laboratory has concentrated on the generation of an FRDA mouse model to investigate the effect of novel therapeutic agents. We have also established fibroblast and neuronal stem cell cultures derived from this model that can similarly be used to investigate novel therapies. With evidence that GAA repeat expansions produce a heterochromatin-mediated FXN gene silencing effect, epigenetic mechanisms, such as DNA methylation, have been investigated with a view to future epigenetic-based therapy. We have recently used bisulfite sequencing technology to assign differentially methylated CpG sites within the GAA upstream region of the FXN gene in human and mouse FRDA tissues. Although this technique is precise, it is also rather labour-intensive and time-consuming. Here we report the development of a MethylScreen technique as a more rapid approach to detecting quantitative DNA methylation changes in the FXN gene. This technique, which involves the digestion of DNA by methylation-sensitive and methylation-dependent restriction enzymes followed by quantitative PCR, has been validated by comparison with our previous bisulphite sequencing data. We further describe the use of this technique to analyse DNA methylation changes that are induced by epigenetic-based therapies within cultured cells and tissues from our FRDA mouse model.

MUTATIONAL ANALYSIS OF SLC26A4 AND FOXI1 IN INDIVIDUALS WITH HEARING LOSS AND INNER EAR MALFORMATIONS. *E. Orzan[1], E. Leonardi[2], M. Martella[2], C. Morando[2], A. Murgia[2]* 1) Pediatric Audiology Unit, Department of Otolaryngology and Otosurgery, University Hospital of Padua, Padua, Italy; 2) Department of Pediatrics, University of Padua, Padua, Italy.

Mutations in the SLC26A4 gene cause Pendred syndrome (PS) and hearing loss with inner ear malformations, mainly enlarged vestibular aqueduct (EVA) syndrome. Both disorders are recessive but many affected individuals show one or no SLC26A4 mutations. In a recent study EVA syndrome was found in a subject carrying a mutation of SLC26A4 in association with a mutation of FOXI1, gene encoding a transcriptional activator of SLC26A4. We have analyzed the SLC26A4 gene in a cohort of 29 unrelated subjects with age ranging from 3 to 43 years. All the selected individuals, only one subject with PS, presented prelingual sensorineural hearing loss often characterized by a progressive/fluctuating course. 14 had documented EVA, 1 had a Mondini dysplasia and 3 had other malformations. 17 different SLC26A4 variants were detected: 11 missense, 2 splice site and 1 frameshift mutation previously reported in other affected subjects, one known polymorphic variant and two new disease-causing mutations. Both the novel variants, c.600GA (p.Q200Q) and IVS9-1GT are believed to alter the splicing mechanisms. In 38% of the subjects, all but the PS case with EVA, at least one SLC26A4 mutation was present. Biallelic SLC26A4 mutations were found in 27,6% (11/29) of the tested subjects, while 10,3% (3/29) of them resulted to be heterozygote. To investigate other possible mechanisms or genetic causes of this condition in negative or heterozygote individuals we performed a gene dosage analysis on SLC26A4 and searched for mutations of the coding sequence of the FOXI1 gene. This analysis allowed detecting a single, putative disease-causing mutation of the coding sequence of the FOXI1 gene (p.161delN), in a subject with Mondini dysplasia, negative for the SLC26A4 analysis. Although signaling the likely presence of other genes involved in the phenotype, the data we report strengthen the importance of molecular analysis of SLC26A4 and FOXI1 in individuals with hearing loss and EVA syndrome.

***HIF1A* gene polymorphism association study in endometriosis and adenomyosis.** *C. Hu[1], W. Lin[2], M. Wu[5,7], J. Lee[6], E. Tsai[1,6], S. Juo[2,3,4]* 1) Graduate Institute of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan; 2) Graduate Institute of Medical Genetics, Kaohsiung Medical University, Kaohsiung, Taiwan; 3) Department of Neurology, Kaohsiung Medical University, Kaohsiung, Taiwan; 4) Center of Excellence for Environmental Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan;; 5) Graduate Institute of Occupational Safety & Health, Kaohsiung Medical University, Kaohsiung, Taiwan; 6) Department of Obstetrics and Gynecology, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan; 7) Department of Family Medicine, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan.

Background: Endometriosis and adenomyosis are characterized by the presence of endometrial glands and stroma outside the uterine cavity. Hypoxia-inducible factor 1, alpha subunit (*HIF1A*) is a potent stimulant of angiogenesis potentially contributing to the pathogenesis of the two common gynecological disorders. The aim of this study sought to investigate whether the *HIF1A* gene polymorphism is associated with susceptibility to endometriosis and adenomyosis. Methods: We performed case-control studies. A total of eight SNPs were selected for genotyping including six common tSNPs and two functional SNPs. Logistic regression analyses were performed to evaluate the genetic effect with adjustment for age and parity. Results: This study recruited 185 patients with endometriosis, 162 patients with adenomyosis, and 380 controls. We found two significant associations between rs1951795 (intron 1) and rs2301113 (intron 10) for adenomyosis. For rs1951795, the results indicated that genotypes AA carried a higher risk than the reference CC genotype (adjust OR=3.57, P=0.006). For rs2301113, genotypes CC carried a higher risk than the reference AA genotype (adjust OR = 2.39, P=0.015). None of the candidate genes were related to endometriosis. Conclusions: The *HIF1A* gene significantly influences the risk of adenomyosis but not endometriosis.

Career Development in Public Health Genomics. *E. Balkite[1,2], C. Lieber[2], R. Grob[2]* 1) ABQ Associates, Inc, Durham, NC; 2) School of Graduate Studies, Sarah Lawrence College, Bronxville, NY.

Healthcare professionals with education and experience in both public health and genomics now have increasingly diverse career opportunities. Purpose: Yet, how does one create positions in a new field or integrate genomics into a current professional role? Many healthcare professionals are unsure of how to initiate this professional challenge. Methods: To meet this need, the Public Health Genomics (PHG) Certificate Program in the Graduate School of Sarah Lawrence College, Bronxville, NY established a model for career development in Public Health Genomics and made it integral to the curriculum of the certificate program. The PHG curriculum in career development is led by an experienced healthcare professional who is also a Career Development Specialist. The curriculum consists of five facets (1) identification of job topology with a searchable database of Public Health Genomics job categories (2) education in job search skills (3) networking with professional role models and mentors, (4) exercises in professional growth and development, and (5) individual career counseling, virtually and in person. Results: The curriculum has varied success with healthcare professionals depending on their professional roles and career status. Professionals whose goal was to integrate genomics into a current role found (3), (4), and (5) most valuable in meeting their needs. Others, seeking to establish a new role for themselves or a new position evaluated (1), (2), and (5) as most valuable. Conclusions: Healthcare professionals benefit from a flexible framework in career development that allows them to customize the components of the framework to meet their needs. Customization leads to the creation of new fields in Public Health Genomics as well as the integration of genomics into current professional roles.

Unbiased estimation of odds ratios combining genomewide association scans with replication studies. *F. Dudbridge, J. Bowden* Boistatistics Unit, Medical Research Council, Cambridge, United Kingdom.

Odds ratios or other effect sizes estimated from genome scans are upwardly biased, because only the top-ranking associations are reported, and moreover only if they reach a defined level of significance. No unbiased estimate exists based on data selected in this fashion, but replication studies are routinely performed that allow unbiased estimation of the effect sizes. Estimation based on replication data alone is inefficient in the sense that the initial scan could, in principle, contribute information on the effect size. We propose an unbiased estimator combining information from both the initial scan and the replication study, which is more efficient than that based just on the replication. Specifically, we adjust the standard combined estimate to allow for selection by rank and significance in the initial scan. Our approach explicitly allows for multiple associations arising from a scan, and is robust to mis-specification of a significance threshold. We require replication data to be available but argue that, in most applications, estimates of effect sizes are only useful when associations have been replicated. We illustrate our approach on some recently completed scans and explore its efficiency by simulation.

A novel mutation of EXT1 gene in a Korean family with multiple exostoses. *S. Shim[1], S. Sung[1], J. Park[1], D. Cha[1, 2], T. Yoon[1], D. Choi[1]* 1) Genetic Lab Fertil Center, CHA Genaral Hospital, College Med Pochon CHA Univ, Seoul, Korea; 2) Dep. of Ob and Gyn, CHA General Hospital, Pochon CHA University, Seoul, Korea.

A 35-year-old female patient diagnosed clinically as multiple exostosis visited the hospital for infertility evaluation and treatment. She had an operation in pelvis, humerus, tibia and femur in 1993. Extended pedigree analysis showed three out of 4 her siblings and several cousins have suffered from the same disease with a typical autosomal dominant pattern of inheritance. So she wanted a genetic test for her disease before having a child. For mutation analysis, DNAs were extracted from the patient and her brother. All exons and exon-intron boundaries of EXT1 and EXT2 gene were amplified by polymerase chain reactions. The PCR products were directly sequenced and analyzed by ABI genetic analyzer. A single base pair deletion [(2236_2241)delC] in the exon 6 of EXT1 gene was detected in both patient and her brother. Generation of a premature stop codon resulting from frameshift of codons might be a causative of the disease. According to the human genome mutation data base (HGMD), the mutation detected is not previously documented.

Natural history of Machado-Joseph disease: a 10 years observational study described through a Markovian method. *L. Jardim[1, 3], C. Kieling[4], A. Vigo[2], S. Camey[2]* 1) Department of Internal Medicine, Universidade Federal do Rio Grande do Sul, Brazil; 2) Department of Statistics, Universidade Federal do Rio Grande do Sul, Brazil; 3) Medical Genetics Service, Hosp de Clínicas, Porto Alegre, Brazil; 4) Psychiatry Service, Hosp de Clínicas, Porto Alegre, Brazil.

Machado-Joseph Disease (SCA3) is a rare autosomal dominant disorder, affecting 3:100.000 individuals in our region. A progressive motor disability supervenes, with a survival of 21 years after the disease onset. Natural history (NH) studies are hard to be done, since patients enter the study with variable disease durations, and are followed-up at irregular intervals. AIMS: show how the long-term progression of a disease, using all the available data, can be done through a Markovian method, and, by using it, describe the progression of gait ataxia (GA), from a SCA3 long-term cohort. METHODS: SCA3 patients were recruited from 1998 to 2005, and were invited to annual neurological follow-ups up to 2007. GA was described through both a MSG (Zilber et al, 98) and a markovian matrix and graph, called Mean Trajectory Graph (MTG). RESULTS: 156 patients were included: at baseline, age and disease duration were of 40.5 12.6 and 7.7 5.8 years (y); age at onset was of 32.8 10.6. There were 28 losses, 27 deaths and 315 neurological evaluations. Patients were seen during 5 2.4 years, with intervals between follow-ups of 2.5 1.5 years. With the MSG and MTG, GA progressed to stages 1, 2, 3 and 4 in, respectively, 2, 8.5, 19 and 25.5 (MSG) or 21 (MTG) years of disease duration. MSG was unsmooth, showing several unlikely ups and downs partially corrected by a posteriori adjustments. MTG produced a continuous curve without any aid. Markovian matrixes of two subgroups, stratified by their CAGn, showed no differences in GA progression ($^2(10)= 9.14$; $p=0.519$). CONCLUSION: The markovian method described the NH of gait ataxia without any a posteriori adjustment of data, and allowed statistical comparisons between subgroups. Using it, we verified that the CAGn did not interfere with this aspect of NH. We believe that Markovian method will probably be useful in future clinical trials in this disease.

***TBX22* promoter haplotypes influence gene expression and are associated with the prevalence of X-linked cleft palate.** *P. Stanier, G. E. Moore, E. Pauws* Institute of Child Health, University College London, London, United Kingdom.

Cleft palate (CP) is a common birth defect that can occur either as an isolated defect or as part of a syndrome. Whilst there is strong evidence for a genetic basis, environmental factors can influence risk, often making a molecular diagnosis difficult. It has proven easier to identify genetic defects in CP patients with additional recognisable features such as ankyloglossia, which is found in about 80% of X-linked CP (CPX) individuals carrying a *TBX22* mutation. In previous studies we have identified apparently classic CPX families with ankyloglossia but no identifiable mutation. Here we report a new investigation of the *TBX22* promoter region and a previously unsequenced 5 non-coding exon for potentially causative sequence changes in this cohort. Unique sequence variants were not present, although, 5 SNPs and one variable dinucleotide repeat were identified. We investigated the frequency of these polymorphisms in CP only (CPO=80), CP & ankyloglossia (CPA=53) and control (n=296) cohorts. We found significant associations for rs6523677 (OR=2.7, $p<0.01$) and rs41307258 (OR=18.3, $p<0.001$) between CPA patients and controls but not between CPO patients and controls. The other variants showed a similar trend but did not reach significance. The polymorphisms were present as three distinct haplotypes. Haplotype 3 was strongly associated with the CPA group (OR=10.1, $p<0.001$) but not CPO or controls. To investigate the functional significance of the different promoter haplotypes, luciferase reporter constructs for each were tested in transient transfection assays. A statistically significant ($p<0.001$) decrease in promoter activity (approximating empty vector) was found for haplotype 3. We therefore report a strong link between the regulation of *TBX22* gene expression and a pathogenic role for reduced *TBX22* transcript levels, potentially explaining many idiopathic cases with CPX like features. As expected, a proportion of the CPO patients also share the risk haplotype, but this was not sufficient to reach significance. This study therefore, highlights the value of ankyloglossia as a predictive diagnostic feature in X-linked cleft palate patients.

Long Term Imapact of Genetic Testing for Deafness: Phenotypic v/s Genotypic Marital Selection. *A. Pandya[1], K. S. Arnos[2], K. Withrow[1], V. Norris[2], S. H. Blanton[3], W. E. Nance[1]* 1) Dept Human & Molecular Genetics, VCU, VA; 2) Gallaudet University, Washington DC; 3) University of Miami, FL.

Hearing loss (HL) is a common neuro-sensory deficit affecting nearly 3/1000 children. The presence of relaxed selection and intense assortative mating among the deaf, have increased the frequency of the commonest form of recessive deafness in the population (Nance and Kearsey, 2004). In the past, assortative mating has been based on phenotype. However, with the advent of molecular testing, the potential exists for replacing phenotypic with genotypic assortative mating. To assess the impact of genetic testing on spouse selection, we compared 303 deaf probands married before counseling and testing for the connexin (Cx) gene, with 413 unmarried students who are followed longitudinally until they select a partner to determine if knowledge about their own Cx status has any impact on their choice of a partner. We present the comparison of frequency of Cx deafness, phenotypic assortative mating and genotypic assortative mating in 303 married probands with a small number of students (N=49) who have married after genetic testing and counseling. The frequency of phenotypic assortative mating was slightly but not significantly higher in students who married after being tested & counseled. Similarly, among students who have married, the proportion of Cx (+) probands with Cx (+) spouses was higher, even though the frequency of connexin deafness was slightly higher in the probands married before 1997. To test for genotypic assortative mating we compared the observed to expected frequencies of Cx (+) by Cx(+) matings in each group assuming random mating, and given the Cx frequency in each group. Interestingly, an excess of genotypic assortative mating was observed in both groups, but did not reach significance in the student group. A 30% excess in observed frequency of genotypic assortative mating compared to the expected in probands married before Cx was discovered must reflect the results of indirect genotypic assortative mating, in addition to the trend towards direct genotypic assortative mating in the students.

Molecular Analysis of Candidate Genes in Keratoconus Patients from the European Population. *DP. Dash[1], S. George[2], AE. Hughes[3], G. Silvestri[2], J. Jackson[2], D. Frazer[2], E. Heon[4], CE. Willoughby[1]* 1) Centre for Vision Sciences, Queen's University Belfast, UK; 2) Royal Victoria Hospital, UK; 3) Dept. of Medical Genetics, Queen's University Belfast, UK; 4) Dept. of Ophthalmology & Vision Sciences, Hospital for Sick Children, University of Toronto, Canada.

Purposes: Keratoconus (KC; MIM#148300) affects ~1/2000 people worldwide and is a common indication for corneal transplantation. At least 9 chromosomal loci have been reported with mutations detected in visual system homeobox 1 (VSX1; MIM#605020) and superoxide dismutase 1 (SOD1; MIM#147450). There are no studies on SOD1 screening in KC cohort except one reported potential mutation IVS2+50 DEL 7. The purpose of the study was to comprehensively screen SOD1 and VSX1 (including two newly identified exons) in keratoconus patients of European ethnicity. Methods: The patient cohort consisted of 66 unrelated KC patients (40 sporadic KC patients and 26 probands with familial KC) of European ethnicity. Mutational analysis of SOD1 and VSX1 including two newly identified exons (6 and 7) was performed by PCR based direct sequencing methods. Results: In total 12 VSX1 sequence variants were detected in KC patients of which c.432C>G (p.D144E), c.479G>A (p.G160D), c.789C>T (p.S263S) were not seen in 200 control chromosomes. The change p.G160D was seen in two sporadic KC patients. Segregation was not detected for p.D144E which represents a polymorphism. Although predicted to alter VSX1 splicing p.S263S had no effect on transcript processing. Two affected members in a KC family showed a novel silent mutation c.180T>C (S60S) in SOD1 gene which was absent in 200 control chromosomes. Bioinformatics testing predicts this sequence variant could be a potential splicing mutation and further RNA studies are ongoing. We did not find the previously reported deletion in SOD1 gene or any other pathological variants. Conclusions: This is the first study to report the complete screening of SOD1 in sporadic and familial keratoconus. The novel silent mutation (S60S) in SOD1 gene in a keratoconus family requires further characterisation at the RNA level. Mutations in VSX1 and SOD1 play a minor role in the pathogenesis of KC in the European population.

Therapeutic response after two years of Galsulfase enzyme replacement therapy (ERT) in five adult patients with Maroteaux-Lamy Syndrome. *C. Lampe, E. Miebach, L. Arash, E. Mengel, M. Beck* Children`s Hospital, University of Mainz, MAINZ, Germany.

Aims: 2006 Galsulfase (Naglazyme) was approved by the FDA and EMEA as enzyme replacement therapy for patients with mucopolysaccharidosis VI (MPS VI). The objective of this study was to proof safety and efficacy of Galsulfase treatment for adult MPS VI patients. **Methods/Patients:** We studied 5 adult MPS VI patients with attenuated presentation, age 27 to 34 years (median: 32), 2 male and 3 female. Before starting enzyme replacement therapy (ERT), all patients underwent an extensive baseline evaluation, they were re-evaluated after 1 and 2 years of treatment. To obtain consensus on contemporary therapeutic aims we used two goals of treatment to confirm therapeutic response: 1. 6 minute-walk test: improvement of at least 54 mtrs. 2. VC-in (inspiratory vital capacity): Improvement of at least 5% above the baseline value. **Results:** Baseline results: Height 135 to 160 cm (median 152), weight 42 to 70 kg (median: 56). Organomegaly in 4/5. Hearing impairment in 0/5, visual impairment and corneal clouding in 3/5. VC-in 42,2% to 85,4% (median 70,1%), thereof 1 patient had a normal lung function. Abnormalities of cardiac valves in 5/5, no cardiomyopathy. Pathological ECG in 2/5 (LVH). Reduced passive shoulder flexion in 4/5. Walking distance evaluated by 6 minute-walk-test varied between 210 and 492 mtrs (median 390). Stenosis of cervical spine in medical history in 2/5. Audiometric, ophthalmological, and cardiologic results showed no changes within two years of treatment. Organomegaly resolved in 3/4. Orthopaedic results did not improve. After 1 year treatment 3/5 achieved an improvement of 6 minute-walk-test. Our 4 patients with abnormal VC-in reached the therapeutic goal. Consequentially, 4/5 patients showed therapeutic response under ERT. In summary, a number of measures of clinical problems were stable or improved over the 2-year period, though there are some aspects of the disease that may be irreversible or difficult to treat, such as joint stiffness and bone disease. ERT was tolerated well, only 1 mild infusion-associated reaction with urticaria was observed.

Role of GABRG2 gene polymorphism in seizure reoccurrence. *S. Ponnala[1,2], J. R. Chaudhari[3], M. A. Jaleel[3], B. Dilnawaz[3], K. P. Rao[1], Q. Hasan[2]* 1) Dept of Genetics, Osmania University, Hyderabad - 500 007, Andhra Pradesh, India; 2) Dept of Cytogenetics and Molecular Medicine, Kamineni Hospitals, L B Nagar, Hyderabad - 500 068, Andhra Pradesh, India; 3) Dept of Neurology and Neurosuegery, Kamineni Hospitals, L B Nagar, Hyderabad - 500 068, Andhra Pradesh, India.

Gamma aminobutyric acid receptor (GABR) are anion-selective ligand-gated channels and occur in 30-40% of all synapses and use Gamma aminobutyric acid (GABA) as their transmitter. Disruption of GABAergic neurotransmission mediated by GABA has been implicated in epilepsy and the nature of the mutant channel may modulate the response to a given treatment. Major anti epileptic drugs used clinically act as GABAergic neurotransmission enhancers. The present study was conducted to evaluate the GABRG2 C588T gene polymorphism in generalized seizure (GS) and febrile seizure (FS) patients to assess whether it confers any susceptibility to seizures in a cohort of patients. 127 cases of seizures, which included 86 GS and 41 FS patients, who were analyzed in this study for GABRG2 C588T gene polymorphism using PCR RFLP. The allele distribution was statistically significant with T allele being associated with GS in our population (OR=2.0508 (95% CI= 1.0205 to 4.1234); X2 $p<0.05$). When the allele frequencies of GS and FS were combined and compared with controls, it showed significant association of T allele with seizures (OR=2.19 (95% CI=1.02 to 4.68); X2 $p<0.05$). In the present study, TT genotype carriers were high in recurrent seizure group of GS compared to well control GS. Compared to GS, in FS, CT genotype carriers were high in recurrent seizure group. Our data shows GABRG2 588T allele carriers have 2 fold increased risk of developing seizures compared to others, TT and CT genotype carriers of seizure reoccurrence group of GS and FS respectively, are at a higher risk of developing recurrent seizure. As yet, there are no studies reporting the role of GABRG2 C588T gene polymorphism in association with seizure reoccurrence.

Whole-Genome Linkage Analysis of High Grade Myopia in a U.S. Asian Family Dataset. *K. N. Tran-Viet[1], A. Bulusu[1], R. Metlapally[1,2], Y. J. Li[1], T. L. Young[1,2]* 1) Duke Ctr Human Genetics, Duke Medical Ctr, Durham, NC; 2) Duke Eye Center, Durham, NC.

Purpose: Myopia or nearsightedness is caused by excessive axial elongation of the globe. High-grade myopia can predispose individuals to premature cataracts, retinal detachment, and glaucoma. Myopia is highly prevalent in Asian countries, and is considered a serious public health issue in these populations. This study focused on U.S. Asian populations and used a whole-genome linkage panel to confirm existing or map novel high-grade myopia loci. **Methods:** A cohort of 10 U.S. families of Asian descent participated in the study with at least two affected (spherical refractive error of -5.00 diopters (D)) siblings per family. Subject refractive errors of -5.00 D were defined as unaffected. Whole genome single nucleotide polymorphism (SNP) genotyping was performed using the Illumina Linkage IVb Marker Panel (6008 SNPs). Genotype data were checked for Mendelian inconsistencies, and linkage analysis was conducted using high myopia as a qualitative trait. Both two-point and multipoint linkage analyses were conducted using the FASTLINK and MERLIN programs, respectively. **Results:** Parametric multipoint analysis demonstrated an HLOD score > 1.00 on chromosomes 1p, 2p, 5q, 10q, 12q, and 22q with corresponding peak HLOD scores of 1.533, 1.424, 1.344, 1.263, 1.553, and 1.269, respectively. Two-point linkage analyses revealed 32 SNPs with HLOD > 1.3. The highest two-point HLOD scores were recorded for SNPs on chromosomes 5q (rs307195, 1.439) and 12q (rs1163016, 1.434). While loci on chromosomes 1, 2, and 10 are suggestive novel linkage regions, loci on chromosomes 5q (Asian), 12q (Caucasian), and 22q (Ashkenazi Jews) have been previously reported. **Conclusion:** Our results show novel suggestive linkage peaks on chromosomes 1p21.20-1p13.2 (14.9 centimorgan (cM)), 2p13.3-2q12.3 (25cM), and 10q25.1-10q26.13 (27.4cM). Previous reported loci on chromosomes 5, 12, and 22 were replicated. These findings further substantiate genetic heterogeneity for myopia predisposition.

Telomeric Unbalanced Chromosomal Translocation t(9,14)(q34, qter)with Progressive Microcephaly, White Matter Lesions and Virschow-Robin Spaces Dilatation. *L. Perrin[1], A. Aboura[2], E. Pipiras[3], C. Baumann[1], C. Dupont[2], AC. Tabet[2], M. Tardieu[4], A. Verloes[1], B. Benzacken[2], M. Gérard-Blanluet[1]* 1) Clinical Genetics, APHP Robert Debré Hospital, Paris, France; 2) Cytogenetics, APHP Robert Debré Hospital, Paris, France; 3) Cytogenetics, APHP Jean Verdier Hospital, Bondy, France; 4) Neuropediatrics, APHP, Kremlin Bicêtre Hospital, Le Kremlin-Bicêtre, France.

The Virchow Robin Spaces (VRS) are invaginations of the subarachnoid spaces which contain spinal fluid that accompany small vessels as they perforate the surface of the brain. Dilated Virchow-Robin spaces are more frequently found in mental retardation (Rollins et al., 1993; Sotos-Ares et al., 2003). We were not able to any find reports of chromosomic abnormality and dilated VRS. We report the case of a child who presents first with neurological features (progressive microcephaly, delayed milestones, cystic Virchow Robin spaces and white matter lesions) in whom we secondary discovered an unbalanced chromosomal anomaly with deletion 9q34 and trisomy 14qter by telomeric screening. The child had neonatal hypotonia and mental retardation. The walk was achieved after 3 years and the language was severely delayed. Minor malformations were present: cryptorchidia, unilateral megauretere and severe gastro-oesophageal reflux. The dysmorphia was not obvious in early age, but was more characteristic of 9q34 microdeletion later on : flat midface, anteversed nares, tendancy to tongue protuding and prognathism. In this patient, the additional trisomy 14qter does not seem to alter the 9qter deletion phenotype. Unusual neurological presentations of 9q34 deletion have already been reported, periventricular leukomalacia and thin corpus callosum (Stewart et al., 2004; Stewart and Kleefstra, 2007), generalized atrophy with marked deep white matter changes (Dawson et al., 2002). We add a new neurological presentation of 9q34 deletion, with progressive microcephaly, cystic Virchow Robin spaces and severe leukomalacia. This observation reminds us that periventricular leukomalacia can be seen in chromosomal anomalies.

Effect of enzyme replacement therapy on gastrointestinal symptoms in children with Fabry disease: results from FOS - the Fabry Outcome Survey. *R. Parini[1], M. Beck[2], R. Hartung[2], G. Kalkum[2], G. Pintos-Morell[3], U. Ramaswami[4] on behalf of the FOS investigators* 1) Metabolic Diseases Unit, San Gerardo Hosp, Monza, Italy; 2) University Childrens Hospital, Mainz, Germany; 3) University Hospital Germans Trias i Pujol, Badalona, Spain; 4) Addenbrookes University Teaching Hospital, Cambridge, UK.

Background: Gastrointestinal (GI) symptoms are an early and common manifestation of Fabry disease, a progressive, X-linked lysosomal storage disorder that leads to major organ failure and premature death. Currently, there are few long-term data on the effect of enzyme replacement therapy (ERT) on GI symptoms in children. Methods: GI symptoms were assessed in children aged 18 years at entry into the Fabry Outcome Survey (FOS), a large database of patients with Fabry disease. For each symptom, the same cohort of children was evaluated at baseline and after 24 months of ERT with agalsidase alfa (Replagal; Shire HGT). Results: In total, 38 children (24 boys, 14 girls) from 8 countries in FOS were assessed. Mean age (range) at onset of symptoms was 6.9 (1-13) years for boys and 7.6 (2-17) years for girls while mean age (range) at start of ERT was 12.1 (3-18) years for boys and 14.7 (4-18) years for girls. At baseline, the most commonly reported symptom was abdominal pain (73%; 19 of 26 children), followed by diarrhoea (35%; 9 of 26 children), nausea (31%; 8 of 26 children), constipation (28%; 7 of 25 children) and vomiting (23%; 6 of 26 children). After 24 months of ERT, prevalence was markedly reduced compared with baseline for abdominal pain (54% vs 73%, respectively) and vomiting (12% vs 23%, respectively). The prevalence of nausea also decreased from 31% at baseline to 27% after 24 months of ERT. Conclusions: ERT with agalsidase alfa has previously been shown to be beneficial in stabilizing the major manifestations of Fabry disease in adults. The present study confirms that GI symptoms are an early manifestation of the condition, with abdominal pain being the most commonly reported symptom in children. However, these symptoms, which have a major impact on quality of life in children, are reduced or stabilized by ERT.

Association of PDE4D with Asthma. *B. E. Himes[1,2,3,4], A. J. Murphy[2], B. Klanderman[2], R. Lazarus[2], J. Lasky-Su[2], M. F. Ramoni[1,3,4], S. T. Weiss[2,4]* 1) Harvard-MIT Division of Health Sciences and Technology, Harvard Medical School, Boston, MA; 2) Channing Laboratory, Brigham and Women's Hospital, Boston, MA; 3) Children's Hospital Informatics Program, Boston, MA; 4) Harvard Partners Center for Genetics and Genomics, Boston, MA.

Asthma is a common inflammatory airway disease with well-established heritability, affecting over 300 million people around the world. More than 100 genes have been individually associated with asthma or a related phenotype. We genotyped over 500,000 single nucleotide polymorphisms (SNPs) in DNA samples from 359 asthma cases and compared them to genotypes from 846 controls. Cases were part of the Childhood Asthma Management Program (CAMP), a clinical trial of asthma treatment in children. Controls were from the Illumina ICONdb public resource. Only SNPs that passed stringent quality-control filters were used for association analysis (n=516,617). Cases and controls, who were all reported to be Caucasian, were matched using genomic ancestry matching (GEM) to reduce population stratification. Association between asthma and each SNP was measured using the Cochran-Armitage trend test as implemented in Plink. The region of strongest association was in and near the phosphodiesterase 4D (PDE4D) gene in chromosome 5q12. The top SNP in this region had an odds ratio of 0.58 (p-value of 1.5E-07), and nine additional linked SNPs in this region had p-values less than 0.001. These case-control analysis findings were supported by family-based association tests using 387 CAMP cases and their parents. Six of the 10 SNPs that were significant in the case-control analysis had additive model FBAT p-values less than 0.05. Despite the limited number of subjects, two lines of evidence suggest that further studies of the potential role of PDE4D in asthma are warranted.

Mutant heat shock protein HSPB8 induces aggregation and a pro-apoptotic phenotype in distal motor neuropathy. *J. Irobi-Devolder[1], J. Krishnan[2], LC. Almeida-Souza[1], I. Dierick[1], C. Ceuterick-de Groote[3], L. Van Den Bosch[2], JP. Timmermans[4], W. Robberecht[2], P. De Jonghe[5,6], S. Janssens[1], V. Timmerman[1]* 1) Peripheral Neuropathy Group, Molecular Genetics Department, VIB, University of Antwerp, Universiteitsplein 1 B-2610 Antwerpen, Belgium; 2) Laboratory of Neurobiology and Experimental Neurology, University of Leuven; 3) Laboratory of Ultrastructural Neuropathology, Institute Born-Bunge, University of Antwerp; 4) Laboratory of Cell Biology and Histology, Department of Veterinary Sciences, University of Antwerp; 5) Neurogenetics Groups, Department of Molecular Genetics, VIB; Neurogenetics Laboratory, Institute Born-Bunge, University of Antwerp; 6) Division of Neurology, University Hospital of Antwerp, Antwerpen, Belgium.

Missense mutations in the small heat shock protein HSPB8 cause distal hereditary motor neuropathy (distal HMN), a motor disorder of the peripheral nerve resulting in severe atrophy and wasting of distal limb muscles. We previously demonstrated that distal HMN mutations target a conserved hot spot residue in the HSPB8 protein and promote formation of aggregates. We now show evidence of axonal degeneration in patients' skin and nerve biopsy. In patient fibroblasts we found multi-foci aggregation of mutant HSPB8 precipitating into aggresomes upon thermal stress. Rat motor neurons and glial cells transfected with mutant constructs show aggregates and degeneration of neurites. Immunostaining using cell death markers demonstrate that non-neuronal cells appear morphologically normal compared to motor neurons. Our findings also demonstrate that mutant HSPB8 induces subtle cell death, which appears to be less dramatic for dividing cells than for postmitotic neurons. Accumulation of mutant HSPB8 could hamper axonal transport and the overall survival of peripheral neurons.

Identification of STRA6 and SKI Mutations and Sequence Variants in Patients with Anophthalmia and Microphthalmia. *T. R. White[1], T. Lu[1], J. Zhou[1], T. Wang[1], K. N. Tran-Viet[1], R. Metlapally[1], T. L. Young[1,2]* 1) Duke Ctr Human Genetics, Duke Medical Ctr, Durham, NC; 2) Duke University Eye Center, Durham, NC.

Purpose: Anophthalmia and microphthalmia (A/M) are rare congenital ocular malformations presenting with absence of eye structure or abnormally small eyes. A/M can be isolated or syndromic. The genes Stimulated by retinoic acid gene 6 (STRA6) and Sloan-Kettering viral oncogene homolog (SKI) are involved in the retinoic acid pathway, and are implicated with A/M in human and animal studies. The retinoic acid pathway is vital to normal eye development and growth. This study explores the association of these genes in a cohort of subjects with A/M. **Methods:** STRA6 and SKI were screened for sequence variants by direct sequencing of genomic DNA samples from 18 patient subjects with A/M. Four external controls were also screened. Coding regions, intron-exon boundaries, and untranslated exons of the genes screened were sequenced by standard techniques. Derived DNA sequences were compared to known reference sequences from public genomic databases. **Results:** For STRA6, a novel coding non-synonymous single nucleotide polymorphism (SNP) was found in one patient, which results in an amino acid change from glycine to glutamic acid in residue 208. One novel nonsense mutation was found in the same patient. These SNPs were not detected in 89 control DNA samples. For SKI, a known coding non-synonymous SNP (rs28384811) was found in 3 patients, which results in an amino acid change from alanine to glycine in residue 62. However, this SNP was also found in 11/89 controls screened. Four novel coding-synonymous SNPs were observed in the SKI gene. **Conclusions:** The STRA6 mutations reported in this study could play a role in the pathogenesis of A/M development, potentially via the retinoic acid pathway. We can attribute 4% A/M incidence in this cohort to these mutations. Although no SKI mutations were found in this cohort, SKI should not be ruled out as a candidate gene for A/M due to the small cohort size.

Gorlin Syndrome: A substantial proportion of previously missing mutations are large PTCH deletions. *S. Benhamed, S. J. Bale* GeneDx, Gaithersburg, MD.

Gorlin Syndrome (GS), also known as basal cell nevus syndrome, is a rare, autosomal dominant multi-system disorder characterized by large numbers of basal cell carcinomas, keratocysts of the jaw, palmar and plantar pits, typical facial features, and other malformations. Mutations in PTCH cause the disorder, with those leading to premature termination of protein translation strongly predominating. Gene rearrangement, presumably resulting in deletion or disruption of PTCH, has been reported in a few patients. Sequencing of the PTCH gene has been the standard method for mutation identification, with sensitivity found to be no higher than 60% in most studies of patients with a diagnosis of GS. We report results of a systematic search for whole or partial PTCH gene deletion in patients referred to a clinical diagnostic lab with suspected Gorlin Syndrome where direct sequencing failed to detect either a pathogenic mutation or any heterozygous positions. We used multiplex ligation-dependent probe amplification (MLPA) to screen for whole gene and single/multi exon deletion in 51 patients. Of these, 13 were found to be deleted for the entire PTCH gene. In addition, one patient had partial gene deletion involving exons 3 to 24. Deletions were confirmed by real-time quantitative PCR or by oligoarray CGH. Two patients were analyzed by array and each showed a large deletion (4.2Mb and 2.7Mb, respectively) that did not appear to result from rearrangement between segmental duplication loci. In each case, the deleted region included the PTCH gene and other genes involved in recessive disorders (e.g. FANCC). These findings confirm that screening by MLPA or arrayCGH is expected to identify at least an additional 28% patients with GS previously missed by sequence analysis. Oligonucleotide arrayCGH analysis using an array with appropriate probe placement should be recommended in patients meeting criteria for GS, but where sequence analysis fails to identify the causative mutation.

Genotype-Phenotype correlations in Scottish Lonq QT syndrome families. *J. Dean[1], C. Brown[1], D. Walker[1], K. Kelly[1], C. Clark[1], P. Broadhurst[2], A. Choy[3], I. Findlay[4], D. Goudie[5], J. Grieve[1], N. Grubb[6], W. Lam[7], V. Murday[8], M. Porteous[7], T. Pringle[3]* 1) Dept Medical Genetics, Argyll House, Aberdeen, UK; 2) Dept Cardiology, Aberdeen Royal Infirmary, Aberdeen, UK; 3) Dept Cardiology, Ninewells Hospital, Dundee, UK; 4) Dept Cardiology, Royal Alexandra Hospital, Paisley, UK; 5) Dept Medical Genetics, Ninewells Hospital, Dundee, UK; 6) Dept Cardiology, Royal Infirmary of Edinburgh, Edinburgh, UK; 7) Dept Medical Genetics, Western General Hospital, Edinburgh, UK; 8) Dept Medical Genetics, Yorkhill Hospital, Glasgow, UK.

We present genotype and phenotype information for 89 Scottish probands with confirmed or suspected long QT syndrome presenting with presumed arrhythmia or sudden cardiac death without deafness. The Familial Arrhythmia Network Scotland, a new organisation of cardiologists, geneticists and pathologists caring for such patients and their families undertakes clinical assessments in each region. DNA samples are analysed in Aberdeen, where initial genotyping involves screening of KCNQ1 exons 3,5,6,7,8,12,13,15, KCNH2 exons 2,6,7,9,10 and SCN5A exons 23-28, which account for 67% of reported Long QT mutations. Of 89 probands, 44 were excluded from analysis because genotyping was incomplete on the cut-off date 30th April 2008. Of 45 whose initial genetic screening was complete, 25 (55.6%) had mutations, including 2 compound heterozygotes and 1 homozygote. There were 17 KCNQ1 and 10 KCNH2 mutations. Phenotype data (QTc, T wave morphology, symptoms, precipitants and age at onset of first event) were analysed for each proband with a mutation. Information on relatives sharing the same mutation was sought to assess further genotype-phenotype correlations. In heterozygous relatives, fewer symptoms were reported and the corrected QT interval was normal in 6/28 (21%). Certain precipitants of arrhythmia (cold water, noise, sleep) are reported to be associated with mutation in specific genes. This correlation was not universal in our cohort. Our data suggest that genotype cannot be reliably predicted from phenotype, and the occurrence of compound heterozygotes necessitates screening of all three genes in every case.

Genetic susceptibility to invasive pneumococcal disease (IPD). *L. Dumitrescu[1], D. Crawford[1], S. Zimmer[2], R. Lynfield[3], N. Messonnier[4], C. Whitney[4], J. McNicholl[4], J. Lingappa[5]* 1) Center for Human Genetics Research, Vanderbilt Univ, Nashville, TN; 2) Department of Medicine, Division of Infectious Disease, Emory Univ, Atlanta, GA; 3) Emerging Infections Unit, Minnesota Department of Health, St. Paul, MN; 4) Centers for Disease Control and Prevention, Atlanta, GA; 5) Department of Medicine, University of Washington, Seattle, WA.

Invasive pneumococcal disease (IPD) is a leading cause of meningitis, pneumonia and blood-stream infection in children and the elderly. A combination of host genetic, pathogen and environmental factors likely determines host susceptibility. We used population-based Active Bacterial Core Surveillance in Minnesota to identify IPD cases in children 5 years of age, occurring between 1997-2000. The de-identified newborn blood spots of 367 (82%) IPD cases and 734 anonymous age, race and hospital of birth-matched controls were used. DNA extracted from 1,101 stored blood spots was whole genome amplified and 967 usable DNA samples (330 cases) were genotyped for 384 tagSNPs in candidate genes. Half the cases were female (52%) and the majority of cases were either European-American (EA; 73%) or African-American (AA; 17%). Population stratification was evaluated using STRUCTURE prior to testing for allelic association; the final stratified cohort consisted of 179/334 and 53/100 EA and AA cases/controls, respectively. Among EAs, one tagSNP (IL12B intronic rs919766) was significantly associated with IPD at p0.01 (OR=1.9; 95% CI:1.2-3.0). Among AAs, two tagSNPs (3' flanking PTAFR rs905907 and 5' flanking SFTPD rs12219080) were associated with IPD at p0.005 (OR=2.6; 95% CI:1.4-4.9 and OR=0.3; 95% CI:0.1-0.7, respectively). rs919766 and rs905907 are in strong linkage disequilibrium (LD) with intronic SNPs while rs12219080 is in strong LD with nonsynonymous rs4469829, which is monomorphic in EA reference populations. This study has identified putative causal SNPs associated with IPD in AAs; however, replication studies are needed to further evaluate the clinical significance of these genetic associations with IPD.

Differing clinical presentation between a mother and son with the same inherited 22q11.21q11.23 microduplication. *V. Siu*[1], *J. Xu*[2] 1) Division of Medical Genetics, Dept of Pediatrics, London Health Sciences Centre and University of Western Ontario, Canada; 2) Cytogenetics, London Health Sciences Centre and University of Western Ontario, Canada.

A 7 ½ year old boy was referred for medical assessment by an orthodontist with regards to multiple dental anomalies. He had fusion of the primary right central incisor to a supernumerary primary central incisor, bilateral posterior and anterior crossbite, overjet of -5 mm, skeletal class III malocclusion, and retrognathia. He also had moderate developmental delay, hyperopia, and astigmatism. There was no history of nasal regurgitation or hypernasal speech. Physical examination revealed normal growth parameters, heavy eyelids with slightly upslanting palpebral fissures, right epicanthal fold, midfacial hypoplasia, downturned corners of the mouth, and relative brachydactyly. The palate was unusual, described as an ogival palate with retropositioned uvula. G-banding analysis at ~700 band level of the boys peripheral blood showed a subtle duplication of 22q11.21-q11.23. This duplication was confirmed by FISH using the probe D22S75 (Vysis) for DiGeorge/VCF critical region at 22q11.2. The father had a normal karyotype. The mother has apparently the same 22q duplication by G-banding and FISH. She had a history of mild learning disability but had completed a regular high school program. Both parents have a history of oligodontia involving one or more molars, but have none of the orthodontic problems or dysmorphic features seen in their son. Microduplication 22q11.2 has been reported in ~40 cases and is emerging as a new syndrome with variable phenotype. This 22q11.2 region is characterized by the presence of multiple low copy repeats and is prone to recombination. Our case emphasizes the need for caution in clinical prediction and genetic counselling for an inherited cytogenetic finding, in view of the possibility of different phenotypes between a carrier parent and child.

Interest in predictive testing for type 1 diabetes among adults. *J. Silver[1, 2], C. Shuman[2], D. Chitayat[2], A. Moore[3]* 1) Molecular Genetics, University of Toronto, Toronto, ON; 2) Clinical and Metabolic Genetics, SickKids, Toronto, ON; 3) Neonatology, SickKids, Toronto, ON.

Objectives: Susceptibility testing for type 1 diabetes (T1D) using a combined genetic and immunological approach is currently available through research studies. Such testing is offered in the context of population-based newborn screening as well as through cascade testing within TID families. It is conceivable that predictive testing for T1D may eventually become available clinically as a public health initiative. Although 1/3 of diagnoses of T1D are made after age 20, little research has been undertaken on the attitudes of adults toward predictive testing for T1D. The aim of this study was to assess interest in T1D testing among those with and without a family history of this condition. Methodology: A self-reporting questionnaire was distributed among two adult cohorts; 203 first-degree relatives (FDRs) of T1D patients and 87 members of the general population (GP) were surveyed. Questions included interest in a test with 100%, 75% and 50% certainty of predicting T1D, perceived risk to develop the disease, and interest in prenatal diagnosis. Results: For a test that has 100% certainty of prediction, 78% of the FDRs and 85% of the GP expressed interest. Interest declined to 51% and 48% respectively if the test was 50% certain. Unexpectedly, self-perceived risk to develop T1D was higher among the GP than the FDRs; a significantly greater proportion felt that they were at higher than 1% risk to develop T1D ($p = 0.005$). Among all subjects, those with higher risk perception ($p = 0.045$) and less education ($p = 0.047$) were more likely to be interested in a 75% certain predictive test. Finally, 48% of the FDRs and 50% of the GP were interested in prenatal diagnosis for T1D. Conclusion: These results suggest that uptake for widespread predictive testing for T1D may be high if the test offers a high positive predictive value. The need for genetic counselling is also highlighted, given that the decision to test oneself or a pregnancy may be mediated by appropriate risk counselling, education regarding test accuracy and exploration of testing motivation.

Public Attitudes about Large Cohort Genetic Research. *D. Kaufman, J. Murphy, K. Hudson, J. Scott* Genetics & Public Policy Center, Johns Hopkins University, Washington, DC.

Introduction: Large cohort studies focusing on interactions between genes, environment, and lifestyle require a large representative sample of the population. In order to recruit and retain participants, it is critical to understand what factors influence support for such a study, and the relative importance of these factors in people's decisions to participate. We surveyed 4,659 Americans aged 18+ about their support for and potential participation in a large prospective cohort study. Methods: A survey instrument, informed by focus groups and community leader interviews that identified key issues, was administered to a representative sample of US residents. To measure the influence of study burden, compensation, and return of research results, respondents were randomized to one of eight study scenarios and then asked whether they would participate. Results: Most respondents (84%) said the study should definitely or probably be done, and overall, 60% would definitely or probably participate given the scenario they were presented. Returning research results to participants (OR=1.6, 95% CI 1.3-1.8, $p<0.0001$) and increasing financial compensation (OR=1.5, 95% CI 1.3-1.7, $p<0.0001$) were both strongly and significantly associated with increased willingness to participate, while a lower study burden was less important (OR=1.2, 95% CI 1.0-1.4, $p=0.015$). The importance of obtaining individual research results was echoed in other survey questions: 75% of respondents would be less likely to participate if research results were not returned and 91% wanted their research results even if there was no immediate clinical value. Support and overall likelihood of participation varied little by demographic group, although the influence of study scenarios on participation differed. Conclusion: Widespread public support exists for a large national cohort study. The prospect of obtaining personal research results can strongly motivate participation, but compensating participants for their time may increase participation by a similar amount. Incentives and recruitment may be tailored to meet different demographics groups interests. This work was funded by a grant from NHGRI.

The proximal and distal human globin CACCC box: a different effect in the hemoglobin switching of the -87 and -101 thalassemia mutations. *M. F. Marongiu[1], S. Porcu[1], D. Poddie[1], D. Drabek[2], T. DeWit[2], A. Cao[1], M. S. Ristaldi[1]*
1) INN-CNR, Cagliari, Italy, Monserrato (CA), Cagliari, Italy; 2) Department of Cell Biology, Erasmus University of Rotterdam, (NL).

The distal CACCC box has been reported not to bind EKLF in vitro. A minor role of the distal CACCC element in globin gene promoter function is suggested by the observation that naturally occurring thalassemia mutations affecting the proximal CACCC box are more severe than those affecting the distal element. Nevertheless recent evidences demonstrate that EKLF binds to the distal CACCC motif, although with low affinity;that the CCTCACCC is required for maximal stimulation of the globin gene by EKLF and that silent thalassemia due to mutations of the distal CACCC box affects the binding to EKLF of the globin gene promoter.We have analyzed in vivo the effect of mutations at the proximal and distal element. We engineered, by site specific mutagenesis, the -101 (distal CACCC element) and -87 (proximal CACCC element) mutations inside the / minilocus construct which contains the full globin LCR, the A globin gene, the globin gene and the 3HS of the globin cluster and produced three mice transgenic lines. The pattern of versus globin switching has been analyzed by S1 analysis and real time PCR. We have dissected the yolk sac at 10 days pc; the fetal liver at 12, 14 and 16 days pc; and the adult blood to assess the embryonic and fetal stages of erythropoiesis. Both mutations appear to be more severe in the adulthood than in the fetal liver stage of development. In addition the pattern of expression of the 101 mutation appears to worsen at day 12 pc than at day 14 pc while the opposite happens in the 87 mutation. This observation is in agreement with the age related pattern of expression of the 101 mutation. In summary the two mutations have a different effect on the activation pattern of the globin gene during the fetal stage of development in transgenic mice highlighting a possible role of the distal globin CACCC box in hemoglobin switching. Our data are in agreement with the observation that both CACCC elements are necessary for optimal globin gene expression.

IL-12B and MCP1 (CCL2) genomic variation associate with pulmonary tuberculosis in two independent West African populations. *D. R. Velez[1,2], G. A. J. Morris[3], C. Wejse[6,7], P. C. Hill[3,4], C. Bisseye[3], R. Olesen[6], M. Sodemann[6], T. L. Edwards[1,2], A. Tacconelli[1], E. Brunetti[1], G. Novelli[1], P. Aaby[7], L. Østergaard[6], W. K. Scott[2], R. A. Adegbola[3], S. M. Williams[5], G. Sirugo[1,3]* 1) Ospedale San Pietro FBF, Rome, Italy; 2) Miami Institute of Human Genomics, University of Miami, Miami, FL; 3) MRC Laboratories, Banjul The Gambia; 4) University of Otago, School of Medicine, New Zealand; 5) Center for Human Genetics Research, Vanderbilt University, Nashville, TN; 6) Department of Infectious Diseases, Aarhus University Hospital, Skejby, Denmark; 7) Bandim Health Project, Danish Epidemiology Science Centre and Statens Serum Institut, Guinea-Bissau.

Mycobacterium tuberculosis pulmonary disease (PTB) has a significant morbidity and mortality worldwide, particularly in sub-Saharan Africa. Immune responses to TB are known to include proinflammatory cytokines and chemokines. The purpose of this study was to examine whether polymorphisms in two of these, interleukin-12B (IL-12B) and monoctye chemoattractant protein 1 (MCP1), were associated with susceptibility to PTB in two West African populations (The Gambia and Guinea Bissau). Eight SNPs were examined in 321 PTB cases and 346 controls from Guinea Bissau and 286 PTB cases and 280 controls from The Gambia. Single locus tests revealed associations in both populations at the IL-12B locus at rs3212227 (OR = 0.78, CI [0.61-0.99], p = 0.05) in Guinea Bissau and rs11574790 (OR = 0.76, CI [0.58-1.00], p = 0.05) in The Gambia. IL-12B haplotypes also associated in Guinea Bissau (global p = 0.0014). The MCP1 promoter variant (rs1024611) only associated in Guinea Bissau (OR = 0.71, CI [0.52-0.97], p = 0.03); these results are concordant with differential susceptibility previously described by Flores-Villanueva et al. (2005) in Mexican and Korean subjects. MDR analyses revealed a significant 3-marker association between MCP1 and 2 markers in IL-12B in Guinea Bissau but not in The Gambia. These data provide evidence for the role of IL-12B in PTB in two distinct West African settings, confirm that an MCP1 promoter variant influences host susceptibility, and suggest an interaction between IL-12B and MCP1 affecting disease risk.

Somatic Second Hits Explain the Localized Nature of Dominantly Inherited Glomuvenous Malformations (GVMs). *M. Amyere[1], V. Aerts[1], P. Brouillard[1], L. Boon[1,2], O. Enjolras[3], M. Wassef[3], JB. Mulliken[4], M. Vikkula[1]* 1) Lab Human Molec Genetics, de Duve Institute, Brussels, Belgium; 2) Centre for Vascular Anomalies, Plastic Surgery, Cliniques Universitaires, UCL, Saint- Luc, Brussels, Belgium; 3) Consultation des angiomes et Cytologie pathologiques, Hôpital Lariboisière, Paris, France; 4) Vascular Anomalies Center, Childrens Hospital and Harvard Medical School, Boston, USA.

Glomuvenous malformations (GVMs) are localized cutaneous vascular lesions, often hereditary and transmitted as an autosomal dominant disorder. We mapped the causative gene to 1p22.1 and identified that the glomulin gene is mutated in almost all familial cases (Brouillard et al., 2002, 2005 and 2008). A second-hit, a 5-bp-deletion resulting in a premature stop codon, was identified in one GVM. This would lead to a complete localized loss of function of glomulin (Brouillard et al, 2002). In this study, pairwise LOH and copy number analyses were performed between blood and tissue using Affymetrix GeneChip 250K and sequencing. Ten somatic mutations were identified: six consisted of the complete deletion of the short arm of chromosome 1, one was a limited 1p22.1-22.2 deletion, one a small intragenic deletion and 2 were intragenic substitutions. In parallel, glomulin expression was examined during murine development using RNA in situ hybridization. Known vascular smooth muscle cell (vSMC) markers (desmin, h-caldesmon, smooth muscle myosin heavy chain and smoothelin-b) were analyzed. Expression of glomulin appeared in vSMCs after desmin, h-caldesmon and smooth muscle myosin heavy chain, but before smoothelin-b. All the markers were expressed in VMs, whereas in GVMs, glomus cells did not express glomulin or smoothelin-b, although smooth muscle myosin heavy chain and h-caldesmon were detected. These data demonstrate that GVMs are caused by a localized complete loss of glomulin, due to the combination of a germline and a somatic second-hit mutation, which cause a deviation in vascular smooth muscle cell differentiation. This explains the variable number, size and localization of lesions (Miikka.vikkula@uclouvain.be, www.icp.ucl.ac.be/vikkula).

Identification of the mutated gene responsible for a new form of Recessive Spastic Ataxia with frequent Leukoencephalopathy (ARSAL) in the French-Canadian Population. *I. Thiffault[1], M. Tetreault[1], V. Bayat[5], L. Loiselle[1], J. Mathieu[2], J. P. Bouchard[3], J. Lesage[4], H. J. Bellen[5], B. Brais[1]* 1) Neuromics Center for Excellence of Université de Montréal, Université de Montréal, CR-CHUM hopital Notre-Dame, Montreal, PQ, Canada; 2) Carrefour de la Santé de Jonquière, Saguenay, QC, Canada; 3) Department of Neurology, CHUL, Quebec City, QC, Canada; 4) Department of Radiology, CHUM-Notre-Dame, Montreal, QC, Canada; 5) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, USA.

Recessive ataxias are a heterogeneous group of neurodegenerative disorders characterized by progressive gait ataxia often associated with pyramidal spasticity. We have recruited a cohort of 28 affected individuals and their unaffected relatives belonging to 23 unrelated French-Canadian families with an original form of Autosomal Recessive Spastic Ataxia with frequent Leukoencephalopathy (ARSAL,SPAX3; OMIM611390). In 2005, using a linkage mapping strategy with a 7cM-genome wide scan (GWS) we mapped the disease locus to chromosome 2q33-35. In order to better define the candidate region, SNP mapping was undertaken using Illumina HAP300DUO microarrays. Homozygosity mapping and linkage analysis confirmed that all ARSAL families are linked to the same 0.54kb candidate region. Haplotype analysis suggests that at least four mutations are present in Quebec. Amplification and sequencing uncovered three insertion mutations in the ARSAL gene. These mutations lead to abnormal size protein on Western blots. The gene codes for a mitochondrial protein. The identification of the mutated gene responsible for ARSAL further underlies the importance of normal mitochondrial function in cerebellar neuron survival.

***Slc7a9* knockout mouse used for searching new potential antilithiasic thiol drugs to treat cystinuria.** *M. Font-Llitjós[1,2], M. Espino[1], L. Feliubadaló[1], R. Sillué[1,2], S. Mañas[1], J. Visa[3], M. Palacín[4,5,6], V. Nunes[1,2,7]* 1) Med & Molec Genetics Ctr, IDIBELL, L'Hospitalet de Llobregat, Spain; 2) CIBER de Enfermedades Raras (CIBERER-U730), Barcelona, Spain; 3) Animal Facility Service, IDIBELL, L'Hospitalet de Llobregat, Spain; 4) Department of Biochemistry and Molecular Biology, Universitat de Barcelona, Spain; 5) Parc Científic de Barcelona, Spain; 6) CIBERER-U731, Barcelona, Spain; 7) Genetic Unit, Department of Physiology II, Universitat de Barcelona, Spain.

Cystinuria is a hereditary disorder caused by a defect in the apical membrane transport system for cystine and dibasic amino acids in renal proximal tubules and intestine, resulting in recurrent urolithiasis. Mutations in *SLC3A1* and *SLC7A9* genes, that encode rBAT/$b^{0,+}$AT transporter subunits, cause cystinuria. In humans, cystinuria treatment is based on prevention of calculi formation and its dissolution or breakage. Persistent calculi are treated with thiol drugs, such as D-penicillamine and tiopronin, for cystine solubilisation. The *Slc7a9* knockout model was used to study the therapeutic effect of 6 potential antilithiasic drugs to treat cystine lithiasis. All drugs are already used for other prescriptions in humans: succimer, sodium thiosulfate, unithiol, uromitexan, tiopronin and D-penicillamine. An in vitro precipitation test and a palatability test were performed for all drugs before performing 3-week treatments of lithiasic mice. Lithiasis evolution throughout drug treatments was followed-up with a non-invasive indirect method of calculi quantification based on densitometry analysis of stones in X-ray plaques. Urine was collected in metabolic cages to quantify amino acid excretion in treated and untreated mice. Histopathological analysis showed that treated lithiasic mice had similar urinary system damage than untreated mice indicating that these drugs had no toxic effects at short exposures. In vivo treatment with all drugs except for STS significantly prevented calculi growth and lowered urinary cystine excretion. Further studies need to be done to assess efficacy and toxicity of these drugs. Acknowledgements: MEC (BFU2006-14600-C02-02); Laboratorios Rubió, SL.

Partial rescue of the adrenocortical dysplasia embryonic phenotype by p53 deficiency. *C. Keegan, B. O'Connor, M. Morley, C. Vlangos, A. Krause* Univ Michigan, Ann Arbor, MI.

Adrenocortical dysplasia (*acd*) is a spontaneous autosomal recessive mouse mutation that exhibits a pleiotropic phenotype with perinatal lethality. The embryonic *acd* phenotype consists of caudal truncation, vertebral segmentation defects, hydronephrosis, and limb anomalies, resembling malformations observed in humans with caudal regression syndrome and VACTERL association. The *Acd* gene encodes a telomeric protein (also known as TPP1) that functions in a multiprotein complex to maintain telomere integrity. Consistent with this function, *acd* mutant mice have evidence of telomere dysfunction and genomic instability. To further explore the relationship between telomere dysfunction and embryonic malformations, we have investigated potential mechanisms leading to caudal dysgenesis in *acd* mutant embryos. Based on the proposed function of Acd as a telomeric protein, we hypothesized that the loss of cells in the caudal region might be due to apoptosis or cell cycle arrest via activation of p53. We observed a significant increase in the number of apoptotic cells in *acd* embryos, but no gross differences in proliferation, suggesting that apoptosis is the predominant mechanism leading to caudal truncation. To determine whether the increased apoptosis is p53-dependent, we crossed *acd* mice to p53 null mice and analyzed the phenotype of progeny from double heterozygote intercrosses. We found that the p53-deficient state does not rescue the embryonic lethality of the *acd* mutation on the C57BL/6 strain. However, a Mendelian genotype distribution was observed at embryonic day 15.5-16.5, suggesting perinatal lethality. Interestingly, the vertebral anomalies were completely rescued in homozygous double mutant embryos. Moreover, the limb hypoplasia phenotype was rescued by p53 haploinsufficiency while complete p53 deficiency in *acd* mutant embryos resulted in preaxial polydactyly. These findings support the hypothesis that the skeletal anomalies in *acd* embryos are secondary to p53-dependent apoptosis. This work will shed important insights into the role of telomere stability during development. This work was supported by a March of Dimes Basil OConnor Award.

miR-93, miR-98 and miR-197 regulate expression of tumor suppressor gene FUS1. *L. Du[1], J. Schageman[1], L. Prudkin[3], I. Wistuba[3], S. Hammond[2], L. Ji[3], J. Roth[3], J. Minna[1], A. Pertsemlidis[1]* 1) The University of Texas Southwestern Medical Center, Dallas, TX; 2) The University of Texas M. D. Anderson Cancer Center, Houston, TX; 3) University of North Carolina, Chapel Hill, NC.

FUS1 is a tumor suppressor gene located on human chromosome 3p21.3. Over-expression of FUS1 significantly inhibits tumor growth and progression in mouse models of lung cancer. Here we show that the 3UTR of FUS1 play a significant role in regulating the Fus1 protein expression and function. Based on computational methods, we predicted that FUS1 is a target of three miRNAs, miR-93, miR-98 and miR-197. By luciferase reporter assay, we validated the interaction of the three miRNAs with the 3UTR region of the FUS1 transcript. By over-expressing FUS1 in NCI-H1299 cells, we demonstrated that individual deletion of the three miRNA target sites in the 3UTR restores the expression level of Fus1 protein. Furthermore, we found that two of the miRNAs, miR-93 and miR-98, are over-expressed across small cell lung cancer cell lines (SCLC) relative to non-small cell lung cancer cell lines (NSCLC) and immortalized human bronchial epithelial cells (HBECs). We also found that elevated miR-93 and miR-197 expression is correlated with reduced Fus1 expression in NSCLC tumor specimens. These results suggest that the three miRNAs play a role in the development of lung cancer by regulating expression of Fus1.

Confirmation of the X-linked "Pierre-Robin sequence and faciodigital anomalies" syndrome. *P. BLANCHET, J. PUECHBERTY, A. SCHNEIDER, M. TOURNAIRE, AM. CHAZE, G. LEFORT, P. SARDA* Service de Genetique, CHU Montpellier, Montpellier, Herault, France.

In 1991, Chitayat et al. described a new apparently X-linked recessive syndrome in 2 half brothers, aged 6 months and 4 years, with the same mother. Patients had Pierre-Robin sequence, facial dysmorphism (retrognathia, high and broad forehead, cleft palate and glossoptosis) and digital anomalies (tapering fingers, hyperconvex nails, clinodactyly of the fifth fingers, narrow distal phalanges, finger-like thumbs and easily subluxated first metacarpophalangeal joints). Both had normal psychomotor development. This entity is probably very rare as no other case has been reported to date. We describe a new patient with a similar phenotype. The boy was born with intra uterine growth retardation, Pierre-Robin sequence, digital anomalies and micropenis. Course was marked by respiratory and feeding difficulties and psychomotor delay. Cerebral MRI was normal. Chromosomal analysis showed an abnormal karyotype: 46,X,der(Y).ish der(Y)t(X;Y)(p22.3;q11.2)(wcpX+,wcpY+,subtelXp/Yp++,subtelXq/Yq-,SRY+,Yq12-),der(X)(p22.3;q11.2)(wcpX+,wcpY+,subtelXp/Yp+,subtelXq/Yq+,SRY-,Yq12-).The karyotype analyzed by classical and FISH methods suggested that the der(Y) chromosome was the unbalanced product of translocation of Xp material onto Yq. The der(X) chromosome was interpreted as the unbalanced product of translocation of Yp onto Xp. These anomalies lead to partial Ypter trisomy and partial Xp disomy in the male patient. Further characterization of this chomosome anomaly by FISH and microarray is ongoing. This observation of a second family with a boy with Pierre-Robin sequence and faciodigital anomalies and a translocation between the X and Y chromosomes confirms this rare X-linked recessive syndrome. Psychomotor delay and micropenis are probably related to the chromosome anomaly.

Experimental approaches to the Imprinted status of the murine *Atp10a* locus. *A. J. DuBose, K. A. Johnstone, J. L. Resnick* Molecular Genetics and Microbiology, College of Medicine, University of Florida, Gainesville, FL.

Prader-Willi syndrome (PWS) and Angelman syndrome (AS) can each be caused by loss of parental contribution from a cluster of genes on chromosome 15. PWS is characterized by a loss of paternal gene expression from this cluster, while AS can be caused by a loss of maternal gene expression from the same region. Regulation of both maternal and paternal gene expression in this region is controlled by a bipartite regulatory element composed of the Angelman syndrome imprinting center (AS IC) and the Prader-Willi syndrome imprinting center (PWS IC). *ATP10A* encodes a putative phospholipid translocase and has been implicated in fat metabolism. The human *ATP10A* locus is a preferentially expressed from the maternal allele and is under the control of the Angelman Syndrome imprinting center. The imprinted status of the orthologous gene in mice, *Atp10a* (*Atp10c, pfatp*), remains uncertain. Deletions including *Atp10a* have been shown to cause increased body fat when inherited maternally, but have no effect when inherited paternally which is consistent with imprinted expression. Molecular studies examining allelic expression have yielded contradictory results. One study showed equal expression of both alleles in all tissues examined while another study found predominantly maternal expression in the olfactory bulb and hippocampus. We are using strain specific polymorphisms in combination with imprinting center mutations to investigate the potential imprinted status of the *Atp10a* locus as well as the role of PWS genes in *Atp10a* expression.

Case report: Cytogenetic abnormalities in a rare CD4+/CD56+ hematodermic neoplasm. *s. michalowski, A. Gerling, F. Johnson, C. Thompson, C. Rehder, M. Selim, B. Goodman* Pathology, Duke, Durham, NC.

CD4+/CD56+ hematodermic neoplasm is a rare and aggressive malignancy of controversial origin. Some research suggests that it is a version of blastic NK leukemia/lymphoma with a cutaneous presentation, while other studies indicate a monocytic, perhaps dendritic origin. The unusual, primary cutaneous presentation, good initial response to therapy and high rate of relapse are hallmarks of this neoplasm. In our case a 12 year old girl presented with a small bruise-like lesion on her lower leg that did not resolve over the course of several months. Immunohistochemistry on a punch biopsy was positive for CD4, CD43, CD56 and TdT, a finding consistent with hematodermic neoplasm. PCR for B cell and T cell clonality was negative, also consistent with this diagnosis. Chromosome analysis of a punch biopsy of the region showed a complex karyotype that included loss of material from 4q, 6q and 9q, plus a gain of chromosome 21 (47,XX,add(4)(q21),del(6)(q23),add(8)(q24.1),del(9)(q22q34),+21[cp3]). Further studies did not show involvement of the bone marrow or cerebrospinal fluid. Currently, there is a lack of, but accumulating chromosomal data on these cases in the literature. Our findings may help to further define genetic changes in this rare tumor.

Identifying a Diagnostic Classifier of CdLS by a Stepwise, Multi-platform Procedure. *Z. Zhang[1], J. Liu[2], D. Wilson[5], M. Kaur[2], M. Deardorff[2,4], D. Clark[2], E. Rappaport[3], M. Morrow[5], I. Krantz[2,4]* 1) Center for Biomedical Informatics, The Childrens Hospital of Philadelphia, Philadelphia, PA; 2) Division of Human Genetics, The Childrens Hospital of Philadelphia, Philadelphia, PA; 3) Nucleic Acid/Protein Core Facility, The Childrens Hospital of Philadelphia, Philadelphia, PA; 4) The University of Pennsylvania School of Medicine, Philadelphia, PA; 5) Xceed Molecular, Toronto, Ontario.

Cornelia de Lange Syndrome (CdLS) (OMIM 122470) is a dominant disorder of multiple congenital anomalies including characteristic facial features, upper limb defects, growth and cognitive retardation and other systemic abnormalities. Although mutations in the NIPBL, SMC1A or SMC3 genes can be identified in about 60% of CdLS probands, molecular diagnosis is not available for the remaining patients. All three causative genes encode subunits or regulators of the Cohesin complex, indicating the existence of a general, Cohesin-related mechanism in CdLS. In this study, we performed a genome wide screen to identify a gene expression profile for CdLS under the hypothesis that defects in Cohesin complex may have a common effect at the gene expression level. We started the screen using comprehensive human genome microarrays (Affymetrix Inc.) and refined it using focused arrays (Xceed Molecular).

Massive parallel bisulfite sequencing of CG-rich DNA fragments. *M. Zeschnigk[1], M. Martin[2], G. Betzl[3], S. Gross[1], K. Buiting[1], B. Frey[3], S. Rahmann[2], B. Horsthemke[1]* 1) Institut für Humangenetik, Universitätsklinikum Essen, Germany; 2) Bioinformatics, Computer Science 11, TU Dortmund, Germany; 3) Roche Diagnostics GmbH, Penzberg, Germany.

Methylation of CpG islands (CGIs) plays an important role in gene silencing, both in normal and disease states. Although next generation sequencers allow massive parallel sequencing, the analysis of the complete human genome in a single run is not yet possible. Guided by *in silico* restriction digests, we used a mixture of four enzymes and a size selection step to generate pools of 350-800 bp DNA fragments enriched for CGIs from female white blood cells and from sperm. The fragments were ligated to adaptors, bisulfite treated and subjected to a modified Roche Genome Sequencer protocol. Using one plate, we obtained 163,034 and 129,620 reads from blood and sperm, respectively, with an average read length of 133 bp. Bioinformatic analysis by newly developed algorithms revealed that 12,358 (7.6%) blood library reads map to 6,167 different CGIs, of which 1,409 (23%) were partially or fully methylated. Almost the same proportion of reads (7.9%) from the sperm library maps to 5,796 different CGIs, of which 820 (14%) CGIs were methylated. CGIs that were hit by reads from methylated and unmethylated fragments are regarded as differentially methylated and were observed in 63 and 53 CGIs in the blood and sperm library, respectively. Significant differences between both samples were found for X chromosomal CGIs. Only 2 of 108 (2%) X chromosomal CGIs that were hit by reads from the sperm library, but 81 of 154 (53%) X chromosomal CGIs that were hit by blood library reads are methylated. We verified the results of eight fully methylated and three differentially methylated CGIs by conventional bisulfite sequencing and found complete concordance. Our results show that the extraction of CG-rich fragments and massive parallel sequencing make it possible to detect tissue and allele-specific methylation differences.

Rare Presentation of Familial Head and Neck Paraganglioma without Evidence of A Mutation in the SDH, RET and VHL genes: Towards Further Genetic Heterogeneity. *A. Persu[1], M. Amyere[2], I. Gutierrez-Roelens[2], P. Rustin[3], C. Sempoux[1], FE. Lecouvet[1], BE. Van beers[1], Y. Horsmans[1], JF. De Plaen[1], M. Hamoir[1], M. Vikkula[2]* 1) Nephrology, Pathology, Diagnostic Radiology, Gastroenterology and Otorhinolaryngology Dept, Cliniques Univ St Luc, Brussels, Belgium; 2) Laboratory of Human Molecular Genetics, de Duve Institute, UCL, Brussels, Belgium; 3) INSERM U676, Hôpital Robert Debré, Paris, France.

SDH mutations in genes encoding succinate dehydrogenase and its anchoring subunits (SDHgenes) are at the origin of hereditary head and neck paraganglioma (PGL) and a subset of apparently sporadic pheochromocytoma. We describe a family including three patients harbouring bilateral head and neck PGL diagnosed before 25 years of age. Multiple hypervascular hepatic lesions were subsequently discovered in two of them. In both, liver biopsy confirmed the diagnosis of PGL. In addition, in one patient, MRI disclosed multiple target-like lesions of the spine, highly suggestive of metastatic PGL. Family history was compatible with autosomal dominant inheritance with possible maternal imprinting. Combined SSCP and heteroduplex analysis followed by sequencing did not show any mutation of the coding parts of SDHB, SDHC, SDHD, RET or VHL genes. Furthermore, succinate dehydrogenase activity measured in a liver PGL sample was not significantly decreased in the affected patients as compared to controls, underscoring the exclusion of the SDH genes. Screening of copy number alterations and LOH using Affymetrix 250K SNP chips in the three affected family members showed no deletion or amplification of the SDH, RET and VHL genes either. No other linked copy number deviation was identified elsewhere in the genome. To our knowledge, this is the first reported family of hereditary head and neck paraganglioma with metastatic dissemination in the liver and the spine. A large body of evidence supports the absence of mutations in SDH, RET and VHL genes, which suggests the existence of a yet unknown gene at the origin of this particular form of familial paraganglioma. (Miikka.vikkula@uclouvain.be, www.icp.ucl.ac.be/vikkula).

Functional analysis of osteoporosis pseudoglioma associated missense mutations in *LRP5*. *A. Saarinen[1, 2], U. Lahtinen[1, 3], M. Somer[4], AE. Lehesjoki[1, 3], O. Mäkitie[1, 5]* 1) Folkhalsan Inst Genetics, Biomedicum Helsinki, Helsinki, Finland; 2) Dept of Medical Genetics, University of Helsinki, Finland; 3) Neuroscience Center, University of Helsinki, Finland; 4) Dept of Medical Genetics, The Family Federation of Finland, Helsinki, Finland; 5) Metabolic Bone Clinic, The Hospital for Children and Adolescents, Helsinki University Hospital, Helsinki, Finland.

Background: Mutations in the low density lipoprotein receptor-related protein 5 gene (*LRP5*) have been associated with high and low bone mass. While homozygous *LRP5* mutations cause osteoporosis-pseudoglioma syndrome (OPPG), characterized by severe osteoporosis and blindness, heterozygous mutations have been associated with reduced bone mass. LRP5 functions as a plasma membrane receptor in the Wnt signaling pathway. We previously described *LRP5* mutations in patients with OPPG and/or severe osteoporosis. In this study we further analyzed the role of these mutations in Wnt signal transduction and cellular localization. **Methods:** Mutations were introduced to full length human LRP5-pcDNA3.1 expression vector using site-directed mutagenesis. Wnt signal transduction assays were performed in 293HEK cells using a previously published Wnt-induced canonical signaling assay (Ai etal. 2005). Localization studies were performed in HeLa cells using immunofluorescence staining. **Results:** Three different missense mutations were identified and selected for further studies. Wnt signaling assays indicated that one of the mutations, R570W in exon 8, completely disrupted Wnt signal transduction. The second mutation, R1036Q in exon 14, resulted in partial disruption of Wnt signaling while the third mutation, R925C in exon 12, did not show any alteration in the signaling assays. Localization studies revealed that the R1036Q and R925C mutant proteins were able to reach plasma membrane where as the R570W could not be detected, suggesting that it might be post-translationally degraded. **Conclusions:** We were able to show that some *LRP5* mutations directly impair Wnt signal transduction and cellular transportation while other pathogenetic mechanisms are associated with some mutations.

Clinical and cellular correlations in Chediak Higashi Syndrome. *W. Westbroek, W. Introne, I. Manoli, G. Golas, D. Adams, D. Maynard, M. Huizing, W. A. Gahl* Medical Genetics Branch, NHGRI, NIH, Bethesda, MD.

Chediak Higashi syndrome (CHS) is a rare autosomal recessive disorder caused by mutations in the CHS1 gene, encoding the 430-kDa CHS1 protein with unknown biological function. Many cell types in CHS manifest giant lysosomes and lysosome-related organelles (LRO). Clinical characteristics can include skin, hair and eye hypomelanosis, recurrent infections, a mild bleeding diathesis and late-onset progressive neurological impairment. The clinical spectrum of CHS varies widely; most patients manifest the severe childhood form, with fatal infections and the accelerated phase while only a few suffer milder infections or progressive neurological impairment. We have shown that the clinical phenotype of CHS varies in relation to the molecular genotype. To study the cellular phenotypes in CHS, we cultured fibroblasts and melanocytes from 5 unrelated patients with vastly different clinical presentations and genotypes. Laser scanning confocal microscopy of cultured primary epidermal fibroblasts stained with the lysosomal marker, LAMP2, showed a perinuclear distribution of a few large lysosomes in severely affected patients; fibroblasts from mildly affected patients contained only slightly enlarged lysosomes. Bright field light microscopy and laser scanning confocal microscopy were used to study the localization, morphology and pigmentation of melanosomes. In control melanocytes, melanosomes exhibited a peripheral distribution with accumulation in the dendritic tips while severely affected patients CHS melanocytes had enlarged melanosomes restricted to the perinuclear area; mild CHS melanosomes were much smaller, with a nearly normal dendritic distribution. Western blot analysis with an anti-CHS1 antibody revealed residual CHS1 protein expression in mild CHS cell lines and absent CHS1 expression in severe CHS-1 cell lines. We show for the first time that the cellular phenotype and CHS1 expression level in CHS patients correlate with the molecular genotype and the clinical phenotype.

Regulatory genetics in childhood leukemia. *J. Healy[1], J. Dionne[1], M. Larivière[1], M. Ouimet[1], V. Gagné[1], N. N'Daye[1], P. Beaulieu[1], D. Labuda[1,2], D. Sinnett[1,2]* 1) Div Hematology-Oncology, Sainte-Justine Hosp, Montreal, Qc, Canada; 2) Department of Pediatrics, University of Montreal, Montreal, Qc, Canada.

It is now widely appreciated that complete loss or gain of function of essential tumor suppressor genes and potent oncogenes may not be the only prerequisite for malignant transformation but rather that increased/decreased gene expression levels might be sufficient to disrupt normal cellular function and drive oncogenesis. Accordingly, sequence variation in gene regulatory sequences has gained much importance in the study of complex disorders due to the quantitative effect on gene expression. We proposed a comprehensive study of functional regulatory variation in genes involved in highly controlled cellular processes known to contribute to cancer susceptibility, such as the cell cycle. We focused on cis-acting regulatory polymorphisms (rSNPs) that could disrupt transcription factor (TF) binding and lead to abnormal expression and variation in gene dosage and therefore influence the risk of disease. The proximal promoter region of 197 candidate genes has been screened and a total of 1838 rSNPs were identified. Population genetic studies are being carried out to evaluate promoter genetic diversity worldwide and the functional impact of these rSNP/haplotypes is assessed combining in silico TF binding site prediction analysis and in vitro functional assays. Thus far, the influence of promoter variation on transcriptional activity has been tested for 17 cell cycle genes and 6 DNA repair genes and DNA-protein binding disruption has been validated for several of these rSNPs. Population- and family-based genetic association studies are performed in parallel to evaluate the impact of functional rSNPs in the risk of childhood acute lymphoblastic leukemia (ALL). Our findings demonstrate how the expected variability in expression levels due to regulatory polymorphisms in key metabolic genes can influence the risk of childhood leukemia and contribute to carcinogenesis, and reveal the functional relevance of the study of regulatory genetics in cancer research.

AXIN2, Orofacial clefts and positive family history for cancer. *R. Menezes[1], M. L. Marazita[1], T. G. McHenry[1], M. E. Cooper[1], K. Bardi[1], C. Brandon[1], A. Letra[1], R. A. Martin[2], A. R. Vieira[1]* 1) Dept of Oral Biology, Center for Craniofacial and Dental Genetics, University of Pittsburgh, Pittsburgh, PA; 2) Dept of Pediatrics, Division of Medical Genetics, St. Louis University School of Medicine, St. Louis, Missouri.

Background: Cancer and congenital malformations may occasionally have a common etiology. We investigated if families segregating orofacial clefts (CL/P) presented increased cancer incidence when compared to control families. Methods: We assessed 75 CL/P families and 93 control families of Caucasian ethnicity from Pittsburgh with regards to positive history of cancer. Chi-square and Fisher exact tests were used to determine statistically significant differences between both groups with alpha of 0.05. Then, we performed molecular studies with genes in which mutations have been independently associated with both cancer and craniofacial anomalies in a total of 111 families presenting isolated CL/P from Pittsburgh and St. Louis. Thirteen SNPs in eight candidate genes (AXIN2, CDH1, FGF3, FGF7, FGF10, FGF18, FGFR1 and FGFR2) were genotyped using Taqman chemistry and transmission distortion was analyzed using the Family Based Association Test (FBAT). Results: CL/P families reported positive family history of cancer more often than control families (p=0.0002), and had higher rates of specific cancer types, including colon (p=0.0009), brain (p=0.003), leukemia (p=0.005), breast (p=0.009), prostate (p=0.01), skin (p=0.01), lung (p=0.02), and liver (0.02). Over transmission of a marker allele in AXIN2 was detected in CL/P probands (p=0.003). Conclusion: Families segregating CL/P may have an increased susceptibility for cancer, notably colon cancer. Further, AXIN2, a gene that when mutated increases susceptibility to colon cancer, is also associated with CL/P. Individuals detected at a higher risk for disease predisposition could be able to adopt a better lifestyle avoiding exposure to other risk factors that may interact with the individuals genotype. NIH grants: R21-DE016718, R01-DE016148 and P50-DE016215.

Genetic heterogeneity for hydrometrocolpos in Bardet-Biedl syndrome: about splitting and lumping with the Mac-Kusick Kauffman syndrome. *H. Dollfus[1,2], E. Schaefer[1], M. Durand[1], C. Stoetzel[2], A. Verloes[3], D. Bonneau[4], C. Hamel[5], P. Bitoun[6], S. Finck[7], A. Goldenberg[8], I. Nisand[9], B. Viville[9], S. Hellé[2], JM. Danse[2], B. Doray[1,2], JL. Mandel[10,11]* 1) Medical Genetics Department, Hôpitaux Universitaires de Strasbourg, France; 2) Laboratoire EA3949, AVENIR Inserm, Faculté de Médecine, Strasbourg, France; 3) Service de Génétique, Hôpital Robert Debré, Paris, France; 4) Service de Génétique, CHU, Angers, France; 5) Service d'Ophtalmologie, CHU, Montpellier, France; 6) Service de Génétique, Bondy, France; 7) CH de haguenau, Haguenau, farnce; 8) Unité de Génétique Clinique, CHU, Rouen, France; 9) Service de Gynécologie Obstétrique, CHU, Strasbourg, France; 10) Laboratoire de Diagnostic Génétique, CHU, Strasbourg, France; 11) Collège de France, Paris, France.

Hydrometrocolpos and polydactyly (and occasional heart malformation) diagnosed in the prenatal period or in infancy may raise diagnostic dilemmas especially in departing McKusick Kauffman syndrome (MKKS) and the Bardet Biedl syndrome (BBS). The two syndromes show clinical overlap that dilutes with time because of the additional features of BBS appearing during infancy such as retinitis pigmentosa, obesity, learning disabilities and progressive renal dysplasia. Genotypic overlap also exists as MKKS-BBS6 gene has been previously found mutated in both syndromes. Herein we report on 7 cases, presenting in the neonatal period as MKKS and who are mutated in various BBS genes and not exclusively to the MKKS-BBS6 gene. The other BBS genes found mutated in the patients presenting with hydrometrocolpos are the following: BBS2, BBS10, BBS8 and BBS12. These findings highlights the great value of genotyping patients with hydrometrocolpos and polydactyly for diagnosis, prognosis and genetic counselling purposes. This data raises also intriguing questions about the phenotypic variability of both syndromes especially for the presence (BBS) or not (MKKS) of retinal degeneration and potential link with genetic modifiers of the phenotype already described for BBS for which oligogenic inheritance is well recognized.

Clinical and biological data of a cohort of 70 patients with 22q11 deletion. *E. HAQUET, P. BLANCHET, C. COUBES, L. PINSON, C. CIANNI, C. LABORDE, G. LEFORT, P. SARDA* Service de Génétique, CHU Montpellier, Montpellier, Herault, France.

DiGeorge syndrome is characterised by the association of facial dysmorphy and several malformations : congenital conotruncal cardiopathy, cleft palate or velar insufficiency, kidney anomalies, hypoplastic thymus and parathyroid glands. Clinical course of the patients is marked by neonatal hypocalcemia, susceptibility to infection due to T-cell deficit, short stature and scoliosis. Language and learning difficulties are common. Psychiatric disorders including paranoid schizophrenia have been described in some adult patients. We report data of a cohort of 70 patients managed in the Reference Center of Montpellier in the South of France. Diagnosis was confirmed in all patients by FISH. Patients are aged from one month to 43 years. Congenital cardiopathies are present in 65% of patients, kidney anomalies in 15% and cleft palate or velar insufficiency in 50%. Hypocalcemia is noted in the neonatal period for 20% of patients, but neonatal hypocalcemia is probably underestimated. Severe or persisting hypocalcemia is noted in 6% of infants. T-cell deficit led to no major illness except frequent otitis and pharingitis in the first years of life. Velopharyngeal insufficiency resulted in nasal speech and language difficulties in the majority of patients and required early language therapy. Even if mental retardation is not present in half of the infants, learning difficulties are extremely frequent. Few patients achieve secondary degrees in school. In our cohort, no adults show paranoid schizophrenia.

Instrument for quality self-assessment in provision of genetic counselling. *H. Kaariainen[1], E. Rantanen[2], M. Hietala[2], U. Kristoffersson[3], I. Nippert[4], J. Schmidtke[5], J. Sequeiros[6], H. Skirton[7]* 1) Dept Mol Med, National Public Health Inst, Helsinki, Finland; 2) Med Bioch Genet, University of Turku, Finland; 3) Dept Clin Genet, University Hospital of Lund, Sweden; 4) Dept Hum Genet, Westfaelische Wilhelms-University Muenster, Germany; 5) Inst Hum Genet, Hannover Medical School, Germany; 6) ICBAS and IBMC, University of Porto, Portugal; 7) University of Plymouth, UK.

Assessment of healthcare services is essential to ensure quality. In genetic counselling, assessing the outcomes is a challenge as many of them (e.g. altered sense of control, peace of mind, or ability to plan for the future) are extremely difficult to measure. In Europe the diverse structures of health services create an additional challenge. However, if genetic healthcare is to be audited, appropriate standards and measurable outcomes need to be defined. The EuroGentest project is a 5 year collaboration between many European experts, with the aim to harmonise and standardise genetic testing in Europe. As genetic counselling is part of the testing process, the quality of counselling issues is also in focus. Recommendations have been made about the need for different types of genetic testing to be accompanied by appropriate genetic counselling, to safeguard those being tested. Patient information materials have been created in several European languages (www.eurogentest.org). We present a self assessment tool developed by EuroGentest Unit 3 for genetic services. The tool that can be applied in self assessment across different systems of genetic healthcare has been developed in expert group workshops and discussed in an open workshop during ESHG Conference 2008. The decision was to emphasize the process instead of the outcome. We have devised a set of standards and potential measurable outcomes for genetic counselling, including items from waiting times and physical clinical environment to access to peer support and continuing professional education, supervision of junior staff, and the actual communication with counsellees. During the pilot phase, the tool has turned out to be helpful in external auditing as well as internal quality assessment.

The Informed Consent Process in Genetic Family Studies. *N. Arar[1,2], L. Panoyan[1], S. Lee[1,2], M. Parchman[1,2], P. Noel[1,2], H. Abboud[1,2]* 1) Dept Medicine, Div Nephrology, Univ Texas Health Sci Ctr, San Antonio, TX; 2) South Texas Veterans Health Care System, Sa, TX.

We examined subjects comprehension of the risks, and benefits associated with their participation in genetic family study (GFS). A cross-sectional study design was employed. A short self-administrative questionnaire was provided to 246 participants who were recruited from families enrolled in the Extended Family Investigation of Nephropathy and Diabetes (EFIND) study conducted at UTHSCSA. Participants responded to the questionnaire directly after their enrollment in the EFIND study. The questionnaire consisted of multiple choice questions and focused on the purposes, procedures, benefits and risks associated with their participation in the EFIND study. These questions were formulated to reflect basic information presented to subjects through the consent process. Responses to questions were expressed as percentages placing equal weight on each response. Average comprehension score of individual subject for all questions was calculated. Then, the total average comprehension score of each question was calculated for all participants. The software package, SPSS (v9), was used to analyze quantitative data. Participants were 62.3% female and averaged 35.2 12.7 years old (range: 18-76). Our findings showed that the average comprehension score was 58. About 30% of the participants did not know the name of the study, and 70% did not identify all elements related to the study procedures. The most striking finding was the lack of understanding concerning the social risks associated with participation in EFIND. While 35.1% of participants identified all potential physical risks, only 1.3% could identify all of the social risks. Our findings showed that participants comprehension score was significantly associated with their level of education ($r=0.2216$, $p=0.0062$) and income ($r=0.2214$, $p=0.0156$). Examination of the current system used for protecting human subjects participating in genetic research has highlighted concerns about its overall effectiveness. Future research directed at improving the communication of risks to subjects of low socioeconomic levels is justified.

A common cytokine profile in Fabry, Gaucher and Hunter diseases. *P. Rozenfeld, N. De Francesco, C. Fossati* Dept Immunolgia, Univ Natl de La Plata, Buenos Aires, Argentina.

A common cytokine profile in Fabry, Gaucher and Hunter diseases Rozenfeld P, De Francesco N, Fossati C LISIN, Facultad de Ciencias Exactas, Universidad Nacional de La Plata. Argentina. The pathophysiology of lysosomal storage diseases (LSD) is not well understood, and it is not solely explained by the burden of storage material. Other concurrent pathological mechanisms are elicited, giving together the phenotypic expression of the diseases. Studies in a spectrum of lysosomal storage disorders showed the upregulation of proinflammatory cytokines. To our knowledge, no analysis of cytokine production by real time PCR in immune cells from Fabry, Gaucher and Hunter human patients has been reported. The aim of this work is to study the cytokine expression in these group of LSD. Blood samples from patients with Fabry (n=24), Gaucher (n= 8) Hunter (n= 8) and 20 normal controls were obtained. Mononuclear cells were separated by Ficoll and cytokine RNA expression by qPCR was determined. A significantly higher expression level of mRNA of IL-1 (p=0.02) and TNF (p=0.03) was observed in mononuclear cells from Fabry patients as compared to normal controls. An overexpression of IL-6 and TNF was detected in Gaucher and Hunter cells in comparison to control individuals. No signs of induction of the genes for IL-4 or IFN was observed in any of these disorders. In conclusion, the cytokine profile overexpressed in Fabry, Gaucher and Hunter immune cells is characteristic of an innate immune response and inflammation, however no signs of induction of an adaptive immune response were detected.

Definitive Diagnosis of Glycogen Storage Disease (GSD) by Molecular Analysis. *N. J. Beauchamp, A. Dalton, S. Tanner, M. Sharrard* Sheffield Children's NHS Foundation Trust, Sheffield, United Kingdom.

Background Hepatic GSDs can cause hypoglycaemia, hepatopathy, lactic acidosis and variable myopathy, requiring different treatment. We have established a diagnostic molecular analysis service for GSDs 0, Ia, Ib, III, IV, VI and IX.

Methods 214 patients were referred with a clinical suspicion of a hepatic GSD or with abnormal enzymology. All exons of the respective genes were sequenced from genomic DNA.

Results Molecular analysis confirmed the diagnosis in 136 (64%) patients. In 19 (14%) of these the analysis changed the diagnosis; with a change of inheritance pattern between X-linked and autosomal, in 16. Mutations were identified in 59 (79%) of 75 suspected GSD IX patients: 45 (76%) had X-linked, and 14 autosomal, GSD IX. Analysis of the 31 patients with suspected GSD VI confirmed 9 (29%) identifying mostly missense mutations in exons 16 and 17. The 11 patients analysed further all had GSD IX. GSD III was confirmed in 39 (83%) of 47 patients. 85% of mutations were null and were located throughout the gene. Patients without muscle symptoms all had exon 3 mutations. Three (18%) of 17 patients analysed had *GYS2* mutations confirming GSD0. GSD Ia was confirmed in 14 (78%) of 18 patients but 2 (11%) were re-diagnosed with GSD Ib. Of the 19 patients with suspected GSD Ib, 11 (58%) had mutations. GSD IV was confirmed in 2 (40%) of 5 patients allowing prenatal diagnosis for one couple.

Conclusions Diagnosis of GSD I can be routinely performed by molecular analysis avoiding liver biopsy and reclassifying between Ia and Ib. Enzymology is inaccurate in distinguishing GSD VI and IX and GSD IX inheritance. Molecular analysis has reassigned both suspected GSD VI patients and inheritance in a significant number of patients. Targeted analysis may be possible in GSD VI but not in GSD III. *GYS2* mutations are found in patients with ketotic hypoglycaemia, though rarely. Molecular analysis for GSD gives a definitive diagnosis and allows rapid, non invasive family studies, including prenatal diagnosis and thus has a first line role in the diagnosis of GSDs.

Towards a pharmacological therapy for Mandibuloacral Dysplasia sindrome. *A. Vielle-Canonge[1], F. Gullotta[2], S. Salvatori[2], P. Molinaro[2], F. Lombardi[1], S. Ciacci[2], M. D'Adamo[3], P. Sbraccia[3], A. M. Nardone[2], M. R. D'Apice[1,2], G. Lattanzi[4], N. M. Maraldi[4], G. Novelli[1,5]* 1) Department of Biopathology , University of Tor Vergata, Rome, Italy; 2) Department of Medical Genetics, A.O.U. Policlinico Tor Vergata, Rome, Italy; 3) Department of Internal Medicine, University of Rome Tor Vergata, Rome, Italy; 4) IGM-CNR, Unit of Bologna, c/o Istituti Ortopedici Rizzoli, Bologna, Italy; 5) University of Arkansas for Medical Sciences, Little Rock, AR, United States.

Recently, different groups have demonstrated that the farnesyl transerase inhibitors (FTIs) were able to reverse in-vitro and in-vivo some phenotypic manifestations of progeroid syndromes secondary to LMNA and/or ZMPSTE24 mutations (Hutchinson-Gilford progeria syndrome and restrictive dermopathy). The rationale of this treatment is based on blockage of the toxic effect of the farnesylated forms of prelamin A which in turn is responsible of cellular morphology alterations and genomic instability. In order to verify if this treatment is reproducible also in the mandibuloacral dysplasia (MADA), we studied the cellular effects of FTIs treatment on primary fibroblasts cell lines after 72 hrs at different concentrations (10 - 500 nM) of the drug. We observed that this treatment induces in MADAs cells, an increase of abnormal nuclei in a dose-manner dependent effect. On the basis of these results, we decided to test the effect of two different drugs (bisphosphonates and statins) known to act on the same biochemical pathway at different levels. We treated MAD fibroblasts in a two steps model (24 hrs statins treatment and then 12hrs bisphosphonate in a single dose). This treatment, showed an improvement of the cellular phenotype (reduction of the number of misshapen nuclei and a partial rescue of the heterochromatin organization). Singularly, these drugs were ineffective. All together these results, suggest that FTI treatment is ineffectiveness versus MADA patients, while inhibitors of prenylation pathways could be considered as potential. This work was supported by the AIFA (Italy) and EURO-Laminopathies (Contract LSHM-CT-2005-018690).

A mouse model heterozygous for protein kinase A regulatory subunit type 1A and catalytic subunit A deficiency develops multiple bone lesions. *K. Tsang[1], M. Starost[2], M. Nesterova[1], S. Boikos[1], A. Li[1], M. Harran[1], C. A. Stratakis[1]*
1) SEGEN, PDEGEN, NICHD, NIH, Bethesda, MD; 2) ORS, DVR, NIH, Bethesda, MD.

Carney Complex is a multiple neoplasia syndrome caused by PRKAR1A-inactivating mutations; PRKAR1A is the type 1A regulatory subunit of protein kinase A (PKA). We have shown that prkar1a+/- mice developed a variety of tumors including tail osteotic lesions. We hypothesized that altered catalytic (C) subunit activity may cause PKA dysfunction that leads to these tumors. Prkar1a+/- mice were crossed with animals heterozygous for the main PKA C subunit (Ca) [Ca+/- mice]; 30 R1a/Ca mice were studied (up to 18 months of age) along with control heterozygote or normal animals. R1a/Ca mice had no endocrine or other tumors except an increased number of tail osteotic lesions. Cartilaginous metaplasia/chondromas were only observed in marrow cavities of long bones and vertebral bodies of R1a/Ca mice. Tail lesions from R1a/Ca mice showed higher PKA activity which was accompanied by a significant decrease in Ca expression; thus Ca was not the only C subunit responsible for increased PKA activity. Immunohistochemical and protein expression analysis implicated potential involvement of PKA catalytic subunit gamma (Cg) and x-linked protein kinase (Prkx) in these lesions. By qRT-PCR and immunohistochemical analysis, we demonstrated an increase in runt-related transcription factor 2 (Runx2), which has been shown to be a major factor in the development of osteoblastic cells and bone formation. It is speculated that increased Runx2 leads to uncontrolled proliferation and differentiation of osteoblasts, and results in the development of cartilaginous chondromas. We conclude that Ca deficiency in prkar1a+/- mice prevents the development of all other tumors but not those of the skeleton; the latter are in fact characterized by increased PKA activity that maybe due to alternate C subunits such as Cg and Prkx (and possibly other factors). Increase in Runx2 expression in these lesions suggests an uncontrolled differentiation of osteoblasts in the background of Ca deficiency; these data point to a previously unknown role of PKA C subunit in the control of osteoblastic proliferation and/or differentiation.

The systematic deficit of heterozygote genotypes in Illumina WGA genotyping is largely explained by null alleles.

G. J. te Meerman[1,2], A. de Vries[2], M. Niens[2], M. Bruinenberg[2], E. Oosterom[2], C. Wijmenga[2], P. Nürnberg[3], D. S. Postma[2], G. H. Koppelman[2], S. M. Leal[1], R. M. W. Hofstra[2], Center for Genomics (CCG) University of Cologne D-50674 Köln/Germany 1) Statistical Genetics and Genetic Epidemiology, Baylor College of Medicine, one Baylor plaza, 77030, Houston, TX USA; 2) University of Groningen and University Medical Center, Hanzeplein 1, 9713AZ Groningen, The Netherlands; 3) Center for Genomics (CCG) University of Cologne D-50674 Köln/Germany.

The signal quality for genotype cluster separation in Illumina genotyping arrays can be improved by taking array specific red/green variation into account. The resulting signal to noise ratio is then typically in a range between 9 and 14 and an improved copy number signal is obtained. The genotyping quality is so high that Mendelian errors, even rare ones appearing a single time in large scale investigations, should hardly occur by chance. In trio genotyping data, a null allele can cause a parent to appear homozygous for one allele and a child homozygous for the other allele. Using autosomal data of 320K Illumina WGA data from 460 trio's we identified 7650 loci with 30269 pairs of a parent homozygous for one allele and a child homozygous for the other allele. A clear signal deficit was observed in these loci, although less pronounced than for the male-female X chromosome difference, indicating that the null alleles have no complete loss of signal. Much longer haplotype sharing exists surrounding the haplotype carrying the putative null allele in contrast with control haplotypes, supporting a more recent origin of the null allele. Average Hardy-Weinberg statistics over all SNP's point to a systematic deficit of about 0.1 % or 386,000 heterozygote autosomal genotypes, possibly caused by null-alleles. Comparison of signal strength at the 7650 loci between case and control haplotypes suggests that null alleles explain most of the deficit in the observed systematic deviation from Hardy-Weinberg equilibrium. Based on haplotype similarity correction of genotypes by resetting the null allele to its original allele at detected loci is feasible.

Geographic Ancestry and Genetics Affect Distinct Pathophysiologic Pathways in Spontaneous Preterm Birth: An Explanation of Disparity. *R. Menon[1], D. Velez[2], S. Fortunato[1], S. Williams[2]* 1) Perinatal Research Ctr, Nashville, TN; 2) Ctr Hum Gen Res, Vanderbilt University, Nashville, TN.

Disparity of preterm birth (PTB) rate between African Americans (AA) and Caucasians (C) (18.5% vs. 12.7%, respectively) is unexplained. Genetic variation has been suspected, and in several genes single SNPs associated with PTB may explain some of the disparity. We performed a large-scale analysis of 1432 SNPs in 130 candidate genes from PTB pathways using a case (PTB)-control (normal term study delivery) design to test for patterns of association in AA and C. Maternal and fetal DNA from 370 (172 cases and 198 controls) C and 279 AA (82 PTB and 197 term) were studied. Single locus association analyses on both maternal and fetal samples were performed. Kyoto encyclopedia of genes and genomes (KEGG) was used to group genes into potential biological pathways. The strongest single locus associations differed in the two races in both maternal and fetal DNA samples. In C maternal DNA, the most significant association was in plasminogen activator tissue (PLAT) gene (rs879293) for both allele (p = 2.00x10-3) and genotype (p=2.0x10-6) association with an odds ratio (OR) of 2.80 [CI 1.77-4.44]. The strongest effect in C fetal DNA was observed in the interleukin-10 receptor antagonist gene (rs17121510) for both allele (p=0.01) and genotype (p=3.34x10-4, OR 1.92 [CI 1.15-3.19]). In AA, the most significant associations were in Interluekin-15 (rs10833, allele p=2.91x10-4, genotype p=2.0x10-3) in maternal DNA with an OR=0.30 [CI 0.14-0.62] and in fetal DNA Interleukin-2 receptor B (rs84460, allele p=1.4x10-4, genotype p=6.3x10-4) with an OR 2.32[CI 1.47-3.67]. Pathway analysis also revealed disparity in PTB by ancestry. In C maternal gene variants had a significant excess of associations in complement-coagulation pathway. Whereas, in AA fetal DNA the infection/inflammation pathway had significantly more associations than expected by chance. Overall, the data suggest that PTB in AA is driven by fetal genotype while in C it is maternal genotype. Therefore, although the terminal events leading to PTB may be the same, upstream pathways appear to differ between African Americans and Caucasians.

Glutaric Aciduria, Type 3: Genetic Mapping to Chromosome 7 and Identification of Mutations in *C7orf10*. *E. A. Sherman[1,2], K. A. Strauss[2], M. J. Bennett[3], D. H. Morton[2], E. G. Puffenberger[2]* 1) Ephrata High School, Ephrata, PA; 2) Clinic for Special Children, Strasburg, PA; 3) Children's Hospital of Philadelphia, Philadelphia, PA.

While screening Old Order Amish infants and newborns for glutaric aciduria type 1 (GA1), we identified three healthy children who excreted abnormal quantities of glutaric acid but low or no 3-hydroxyglutaric acid, a pattern consistent with glutaric aciduria type 3 (GA3). None of the three children harbored the *GCDH* c.1262C>T mutation for GA1 that is known to segregate in the Old Order Amish population. We initiated a genome-wide mapping study using Affymetrix 10K SNP Mapping Arrays and identified a homozygous 4.7 Mb region on chromosome 7 common to all three children. This region contained 25 known genes, including *C7orf10*, an open reading frame predicted to contain a mitochondrial targeting sequence and a coenzyme-A transferase domain. PCR and sequencing of the 15 coding exons of *C7orf10* revealed that all three Amish individuals were homozygous for a non-synonymous sequence variant, c.895C>T (Arg299Trp). An additional non-Amish GA3 patient was sequenced for *C7orf10* and was found to be a compound heterozygote for the Amish variant and a second nonsense mutation, c.424C>T (Arg142Ter). Subsequently, a DNA sample from the original GA3 patient described by Bennett et al. (1991) revealed homozygosity for a novel nonsense variant, c.322C>T (Arg108Ter). This small-scale mapping study firmly establishes *C7orf10* as the causative gene for glutaric aciduria, type 3. Future studies that determine the exact biochemical relationship between *C7orf10* and *GCDH* may establish a foundation for more effective treatment of GA1.

Characterization of N-terminal huntingtin Fragments that Accumulate in the Nucleus. *L. R. Smith[1,2], S. H. Li[1], X. J. Li[1]* 1) Department of Human Genetics, Emory University, Atlanta, GA; 2) Graduate Program in Genetics and Molecular Biology, Emory Univ, Atlanta GA.

Huntingtons disease (HD) is an autosomal dominant, late-onset neurodegenerative disorder characterized by the expansion of a polyglutamine (polyQ) repeat located in the N-terminal region of the huntingtin (htt) protein. Wild-type htt consists of less than 36 glutamine repeats whereas the mutant version of the protein has an expanded polyQ repeat of greater than 37 glutamines. Htt is normally a cytoplasmic protein but in HD, N-terminal fragments of mutant htt localize to the nucleus to form inclusions. In the nucleus, mutant htt has been shown to aberrantly interact with various proteins such as the transcription factors Sp1 and TAFII130. The nuclear toxicity of mutant htt is indicated by the fact that mutant htt preferentially accumulates in the nuclei of striatal neurons, which are most vulnerable in HD. However, the mechanism for the nuclear accumulation of mutant htt remains to be investigated. We first aimed to identify the size of the mutant htt fragment that can accumulate in striatal nuclei. We transfected HEK 293 cells with N-terminal htt fragments of various lengths (212, 300 and 500 amino acids). Using immunocytochemistry and nuclear fractionation techniques, we analyzed whether each of the fragments could accumulate in the nuclei. We found that the smaller fragment of 212 amino acids showed more nuclear accumulation than larger fragments of 300 and 500 amino acids. Next we aimed to identify nuclear proteins whose expression is altered by mutant N-terminal htt expression. To this end, we are using an approach known as SILAC (Stable Isotope Labeling in Cell Culture) to compare nuclear protein profiles in HEK 293 cells transfected with N212-htt containing 150Q and 23Q. These studies aim to reveal how mutant htt accumulates in the nucleus and affects nuclear function. Supported by NIH grants NS 045016 and NS41669.

Computing power and sample size for case-control association studies with copy number polymorphism: application of mixture-based likelihood ratio test. *S. Finch[1], W. Kim[2], D. Gordon[3], J. Sebat[4], K. Ye[5]* 1) Dept Applied Math, Stony Brook Univ, Stony Brook, NY; 2) Dept Math and Stat, Univ S Florida, Tampa, FL; 3) Dept Genetics, Rutgers Univ, Piscataway, NJ; 4) Cold Spring Harbor Labs, Cold Spring Harbor, NY; 5) Albert Einstein College Medicine, Bronx, NY.

Background: Recent studies suggest that copy number polymorphisms (CNPs) may play an important role in disease susceptibility and onset. Currently, the detection of CNPs mainly depends on microarray technology. For case-control studies, conventionally, subjects are assigned to a specific CNP category based on the continuous quantitative measure produced by microarray experiments, and cases and controls are then compared using a chi-square test of independence.

Methods/Results: The purpose of this work is to specify the likelihood ratio test statistic (LRTS) for case-control sampling design based on the underlying continuous quantitative measurement, and to assess its power and relative efficiency (as compared to the chi-square test of independence on CNP counts). The sample size and power formulas of both methods are given. For the latter, the CNPs are classified using the Bayesian classification rule. The LRTS is more powerful than this chi-square test for the alternatives considered, especially alternatives in which the at-risk CNP categories have low frequencies. An example of the application of the LRTS is given for a comparison of CNP distributions in individuals of Caucasian or Taiwanese ethnicity, where the LRTS appears to be more powerful than the chi-square test, due to misclassification of the most common CNP category into a less common category.

Conclusions/Significance: Substantial power may be gained by performing case-control analyses on the underlying CNP quantitative measures rather than on categorical data derived from these measures. Our method quantifies this power increase.

P14 GENOMIC AND EPIGENETIC CHANGES: NO CORRELATION WITH DNA MISMATCH REPAIR DEFICIENCY IN COLORECTAL CANCER. *C. Nyiraneza[1], C. Sempoux[2], A. Kartheuser[3], R. Detry[3], K. Dahan[1]* 1) Human Genetics Unit, Université Catholique de Louvain; 2) Department of Pathology, Cliniques Universitaires St Luc, Brussels; 3) Department of Colorectal Surgery, Cliniques Universitaires St Luc, Brussels.

The tumor suppressor gene *p14* is a major sensor of oncogenic stress in mammalian cells. p14 antagonizes MDM2 protein Ub-ligase activity, thereby stabilizes p53 protein. *p14* promoter methylation has been involved in colorectal cancer (CRCs), particularly in microsatellite instability (MSI) CRCs. Here we correlated the genomic and epigenetic alterations at *p14* locus with the molecular nature of DNA mismatch repair system (MMR) defects resulting in MSI, and we compared these data with *p53* status. Ninety eight CRCs were tested for *p14* methylation using Methylation Specific PCR (MSP) completed by BSP cloning and sequencing, for gene dosage and mutations (exon 1 and exon 2) in *p14* gene, as well as for p53 protein immunohistochemistry (IHC) and *p53* mutation analysis. MMR system sufficiency was assessed by MMR proteins IHC, MSI status and *MLH1*/*MSH2* germline mutation screening, *MLH1* promoter methylation and *BRAF* hot spot exon scanning. Overall, *p14* promoter hypermethylation was observed in 7 tumors (7/98; 7.1%). Of them, 3 were MSI-High CRCs due to MLH1 promoter silencing (1/3), and associated with p.V600E *BRAF* activating mutation (1/3), and 4 were microsatellite stable (MSS) CRCs showing a p53 protein overexpression, with two *p53* hotspot mutations [p.R158H, p.R267Q] (1/4). By contrast, both methylated and unmethylated alleles were detected in 91 CRCs (93%), 22 out them were MSI (MSI-High versus MSS, P>0.05), and the same profile was observed in all adjacent normal tissues (100%). Clonal bisulfite sequencing showed a mosaic state of *p14* promoter methylation in tumors and adjacent normal mucosa tested. The distribution of methylated CpG sites among clones was heterogeneous, showing no specific profile. No allelic imbalance was detected, and mutation analysis revealed one somatic mutation (p.A121V) in *p14* gene. Our data showed no significant correlation between *p14* hypermethylation and MSI status in CRCs. *p14* promoter silencing and somatic mutations appear as rare events in colorectal carcinogenesis.

Genetics variants in Sar1B gene associated with Chylomicron retention disease. *R. Sanchez[1], E. Levy[1,2], D. Sinnett[1,3]* 1) Research Center, CHU Sainte-Justine, University of Montreal, Montreal, Canada, H3T 1C5; 2) Department of Nutrition, University of Montreal, Canada, H3C 3J7; 3) Department of Pediatrics, University of Montreal, Canada, H3C 3J7.

Chylomicron retention disease (CMRD) is a rare, autosomic recessive disorder characterized by severe fat malabsorption associated with chronic diarrhea, failure to thrive, and hypocholesterolemia in childhood. The requirement of Sar1B for the intracellular CM transport has only recently been established in view of specific mutations in Sar1B gene. These mutations are directly associated with the disease-related phenotypes. Given the wide range of biochemical and clinical manifestation of CRMD, we suspect the presence of novel Sar1B mutations and/or DNA variants (SNPs). To address this issue we resequenced the Sar1B exons and promoter regions to detect new SNPs that could be associated with CRMD. Genomic DNA from peripheral blood cells was isolated from 14 CRMD patients that have been treated for a fat malabsorption syndrome associated with diarrhea and steatorrhea. PCR products corresponding to the proximal promoter region (2514 bp) and all 6 exons (763 bp) were directly sequenced to detect novel SNPs in Sar1B gene (Ref Seq NM_016103). We identified 10 known DNA variants including 7 promoter SNPs and 3 coding SNPs (cSNPs), two in exon 4 and one in exon 8. None of these SNPs were polymorphic in the group of patients (n=14) investigated. In addition, we also found four mutations (Glu122X, Asp137Asn, Ser179Arg and Gly185Val) known to be associated with CRMD phenotype. The SNP frequency and haplotype structure were also determined. More work is needed to investigate the impact of these Sar1B gene variants on lipid parameters, apolipoprotein profile and clinical features.

Allelic expression of the *MMACHC* gene and genotype-phenotype correlations in *cblC* disease. *N. Anastasio[1,2], J. P. Lerner-Ellis[1,2], T. M. Pastinen[1,2,3], J. Liu[1,2], D. Coelho[4,5], T. Suormala[4,5], M. Stucki[4,5], A. Loewy[1,2], S. Gurd[1,3], E. Grundberg[1,2,3], C. F. Morel[6], M. R. Baumgartner[4,5], D. Watkins[1,2], B. Fowler[4,5], D. S. Rosenblatt[1,2]* 1) Department Human Genetics, McGill University, Montreal, Canada; 2) Department of Medical Genetics, McGill University Health Centre, Montreal, Canada; 3) McGill University and Genome Quebec Innovation Centre, Montreal, Canada; 4) Metabolic Unit, University Childrens Hospital, Basel, Switzerland; 5) Division of Metabolism and Molecular Pediatrics, University Childrens Hospital, Zürich, Switzerland; 6) Department of Medicine, University Health Network, University of Toronto, Canada.

Combined methylmalonic aciduria and homocystinuria, *cblC* type, caused by mutations in *MMACHC* located in chromosome region 1p34.1, results from the inability to convert intracellular vitamin B_{12} (cobalamin) into its two active coenzyme forms. Methylcobalamin is required by methionine synthase in the conversion of homocysteine to methionine, and adenosylcobalamin is required by methylmalonyl-CoA mutase in the conversion of methylmalonyl CoA to succinyl CoA. Individuals with *cblC* disease may present early in life or at a much later age. With regard to genotype-phenotype correlation, individuals with the c.394C>T (p.R132X) mutation generally have late onset of disease whereas patients with the c.331C>T (p.R111X) and c.271dupA (p.R91KfsX14) mutations usually present in infancy. Previous quantitative real-time RT-PCR studies showed increased *MMACHC* mRNA transcript levels in patients homozygous for the late-onset c.394C>T mutation when compared to both controls and patients homozygous for the early onset c.271dupA and c.331C>T mutations. Allele-specific expression analysis was carried out on human *cblC* fibroblasts with compound heterozygous mutations. Increased transcript levels were consistently observed from the c.394C>T allele when compared to the c.271dupA and c.331C>T alleles. Understanding the mechanisms underlying differential *MMACHC* transcript levels may provide a clue as to why individuals carrying the c.394C>T mutation generally present later in life.

Genetic interactions and vision defects of Bardet-Biedl Syndrome chaperonin-like genes in the zebrafish: pair-wise knockdown suppresses Kupffers vesicle phenotype. *L. Baye[1], E. M. Stone[2,3], V. C. Sheffield[3,4], D. C. Slusarski[1]*
1) Dept of Biology, Univ of Iowa, Iowa City, IA; 2) Dept of Ophthalmology, Univ of Iowa, Iowa City, IA; 3) Howard Hughes Medical Institute; 4) Dept of Pediatrics, Univ of Iowa, Iowa City, IA.

Bardet-Biedl Syndrome (BBS) is a pleiotropic, genetically heterogeneous autosomal recessive disease characterized by obesity, retinal degeneration, polydactyly, kidney malformations, hypogenitalism and learning disabilities. Twelve BBS genes have been identified to date and although the precise roles of the BBS proteins are yet to be elucidated, cilia and cilia-related functions appear to be central features of the disorder. To gain insight into the pathophysiology of BBS, as well as potential BBS gene interactions, we are utilizing the zebrafish model system. My work has focused on characterizing phenotypes associated with three identified chaperonin-like CCT family members that underlie approximately 25% of BBS cases: BBS 6, 10 and 12. Upon individual knockdown of these genes using antisense morpholinos, we found defects in the formation of a transient ciliated structure known as Kupffers vesicle as well as delays in melanosome transport. These two phenotypes are the cardinal features of BBS gene knockdown in zebrafish. Previously published work utilizing these two phenotypes determined genetic interactions in a subset of BBS genes, *bbs1-8*. Because members of the CCT family are known to interact as complexes, we tested genetic interactions among the BBS chaperonin-like genes. Interestingly, pair-wise co-injections of low dose BBS morpholinos suggest that BBS6 and BBS10 interact genetically to suppress the Kupffers vesicle defect. In addition, to examine the molecular and cellular pathways underlying vision loss in BBS, we are utilizing vision screening assays specific to the zebrafish. The first assay measures an adaptive response to light in which zebrafish contract pigment. The second assay measures cone visual function by examining a behavioral escape response to a visual stimulus. Vision analyses on morpholino injected embryos indicate reduced visual function with knockdown of BBS12.

EARLY-ONSET SEIZURE VARIANT OF RETT SYNDROME IN A 47,XXY BOY WITH A NOVEL CDKL5 MUTATION. *S. Sartori[1], R. Polli[2], G. Di Rosa[3], E. Bettella[2], G. Tricomi[3], G. Tortorella[3], A. Murgia[1,2]* 1) Pediatric Neurology Unit, Department of Pediatrics, University of Padua, Padua, Italy; 2) Rare Disease Laboratory, Department of Pediatrics, University of Padua, Padua Italy; 3) Infantile Neuropsychiatry, Department of Medical and Surgical Pediatrics, University Hospital of Messina, Messina Italy.

Mutations in the X-linked cyclin-dependent kinase-like 5 (CDKL5) gene have recently been found in patients with severe neurodevelopmental disorders, including infantile epileptic encephalophathy, severe X-linked infantile spasms (ISSX) and mental retardation, autism, and the early-onset seizure variant of Rett syndrome. As part of a clinical and molecular study we are conducting on encephalopaties of the first year of age associated with epilepsy, we have ascertained a child presenting with a severe early-onset epileptic encephalopathy, mild dysmorphic features, global developmental delay, and profound intellectual and motor impairment reminiscent of Rett syndrome. A standard karyotype analysis showed the presence of two copies of the X chromosome (47XXY by ICN 2005). A mutation scanning and quantitative analysis of the MECP2 gene coding sequence resulted negative, while the molecular analysis of the CDKL5 gene allowed the identification of a de novo heterozygous mutation at nucleotide 1675 of the coding sequence (c.1675C>T) resulting in the creation of a premature stop codon (p.Arg559Stop). The pattern of X chromosome inactivation was found to be balanced. This pathogenic CDKL5 mutation, never previously described, truncates the large COOH-terminal region of the gene, crucial for the proper subcellular localization of the CDKL5 protein. We report the second case of intragenic CDKL5 mutations found in a male subject, the first one detected in an individual with 47,XXY karyotype. We would like to draw attention on the importance of considering the causal involvement of CDKL5 in males showing early onset seizures and Rett-like clinical features, as well as other phenotypes that have been related to mutations of this gene in females.

SNP Discovery in High-throughput Resequenced Microarray-Enriched Human Genomic Loci. *A. A. Antipova, T. D. Sokolsky, C. R. Clouser, E. T. Dimalanta, C. L. Hendrickson, C. Kosnopo, C. C. Lee CC, S. S. Ranade, L. Zhang, K. J. McKernan* Applied Biosystems, Beverly, MA.

Identifying genetic variants and mutations that underlie human diseases requires development of robust, cost-effective tools for routine re-sequencing of the regions of interest in the human genome. Here we demonstrate that coupling Applied Biosystems SOLiD^{TM} System sequencing platform with microarray capture of targeted regions provides an efficient and robust method for high-coverage re-sequencing and single nucleotide polymorphism (SNP) discovery in human protein-coding exons. Utilizing high-density Agilent microarrays with a custom probe design to pull down 4.3 Mb target DNA sequence from a HapMap Yoruba sample, we obtained sequencing coverage averaging138 tags per base (median coverage is 59) with 91% of target sequence covered by at least one tag. This level of coverage enabled highly accurate and sensitive SNP detection. 99.9% of identified homozygous HapMap SNPs were called correctly, and 96.2% of heterozygous HapMap SNPs found de novo by us were correctly identified. Additional 35 SNPs, called unknown in HapMap (not defined as either homo- or heterozygous in HapMap), were Sanger-sequenced to verify their identity, and 96% of these SNPs were correctly called by SOLiD^{TM}. On the whole, 3526 SNPs were de novo discovered by SOLiD^{TM} in the target regions, with 73% of these SNPs overlapping positions in dbSNP database. The remaining 27% are novel SNPs. These results demonstrate that the combination of SOLiD^{TM} resequencing with microarray capture of the selected genomic regions provides a powerful tool for genetic analysis and will expedite the search for genes contributing to inherited common diseases and the diseases in which somatic mutations play a role, such as atherosclerosis and cancer.

Fragile X intermediate and small premutation alleles (45 - 69 repeats): Instability on transmission. *S. L. Nolin, A. Glicksman, T. Sukontasup, N. Hosmer, C. Dobkin, W. T. Brown* Human Genetics Dept, NYS Institute for Basic Research in Developmental Disabilties, Staten Island, NY.

We have examined the transmission of 210 alleles in 85 families with fragile X CGG repeats from 45 to 69 identified through population screening. Most (181) of the transmissions were maternal with the remaining 29 paternally transmitted. 40% (72/181) of maternal transmissions were unstable.

Maternal CGG Repeats	No. Unstable/total (%)	Average Repeat Change
45-49	6/26 (23)	1.7
50-54	11/47 (23)	3.1
55-59	30/69 (43)	2.2
60-64	11/22 (50)	5.2
65-69	14/17 (82)	14.4

For alleles with fewer than 65 repeats, most of the expansions were limited to 1 to 6 repeats. The most notable exceptions were an expansion from 54 to 85 repeats and a contraction from 53 to 33 repeats. Most alleles with 65-69 repeats increased by 20 to 27 repeats although some increased by 2 to 5 repeats. No expansions to full mutation were observed among the newly identified families. For the paternal transmissions 30% (9/29) were unstable; 8 were expansions ranging from 2 to 8 repeats and 1 was a contraction of 2 repeats. These results suggest that most alleles identified through population screening exhibit less instability than alleles with the same repeat size identified in families with affected individuals.

Age-related hearing loss is not caused by variants in the connexin genes that are implicated in prelingual deafness. *A. L. Souza[1], P. Libby[1], F. M. Mapes[2], S. T. Frisina[2], D. A. Eddins[2,3], R. D. Frisina[2,3], D. L. Newman[1]* 1) Biological Sciences, Rochester Institute of Technology, Rochester, NY; 2) International Center for Hearing and Speech Research, Rochester Institute of Technology, Rochester, NY; 3) Otolaryngology, University of Rochester Medical Center, Rochester, NY.

Mutations in the connexin genes, which are critical for cochlear gap junctions and K^+ cycling, are the most common cause of nonsyndromic sensorineural deafness. We investigated whether these genes are involved in the related phenotype of presbycusis (age-related hearing loss), a complex, progressive disorder that affects up to half of all elderly Americans. We targeted specifically *GJA1*, *GJB2*, *GJB3*, and *GJB6*, all of which code for connexins localized in the inner ear, and all of which have been associated with prelingual deafness. A screening set of 24 European-American subjects was sequenced for variants in the exon and upstream regulatory regions of each gene. No common amino acid changes were found, though three were observed at low (2%) frequency. We identified 13 variants in *GJA1*, 12 in *GJB2*, 22 in *GJB3*, and 7 in *GJB6*. In the 1 kb upstream region of *GJA1*, 9 rare variants were found, 7 of which were only observed in affected subjects and not in controls. To investigate whether or not variants in the *GJA1* promoter region are important, an additional 100 (50 best and 50 worst hearing) samples were sequenced. All SNPs were found at a low frequency, and there was no significant difference between the two groups. Genotyping of four SNPs in *GJB2* for ~600 European-American subjects indicates that there is no association between genotype and hearing phenotypes in our population. We are continuing to genotype additional SNPs in each of the four genes, but it appears that connexin gene variants do not play a significant role in the development of presbycusis.

Congenital Disorder of Glycosylation Type Ia Due to Paternal Uniparental Isodisomy 16. *G. E. Tiller[1], R. R. Mardach[1, 2], A. Still[3], T. C. Wood[3]* 1) Dept Genetics, Kaiser Permanente, Los Angeles, CA; 2) Dept Pediatrics, UCLA Medical Center, Los Angeles, CA; 3) Greenwood Genetic Center, Greenwood, SC.

Congenital disorders of glycosylation (CDG) are a genetically heterogenous group of inherited metabolic diseases, each affecting the posttranslational processing of glycoproteins. We describe an infant with CDG type Ia, the most common of these disorders, due to paternal uniparental isodisomy for chromosome 16. She was the second child born to a Mexican mother and Cambodian father. She presented at 5 months of age with acute respiratory failure due to bronchiolitis, hepatomegaly, and a history of neonatal apnea, hypotonia, failure to thrive, and microcephaly. Physical exam further revealed growth retardation, inverted nipples, and enlarged vulvar fat pads. Brain MRI revealed cerebral and cerebellar atrophy; liver biopsy revealed severe steatosis with moderate fibrosis. Spectroscopic analysis of serum transferrin exhibited increased asialo and disialo isoforms. Sequence analysis of the phosphomannomutase 2 (PMM2) gene revealed homozygosity for a c.G691A transition, resulting in amino acid substitution p.Val231Met. Due to the relative rarity of this allele and few reported cases of homozygosity for PMM2 mutations, analysis for uniparental disomy (UPD) was undertaken. Genotyping of the patient and parents at eight markers spanning all of chromosome 16 was consistent with paternal uniparental isodisomy. This case represents the first report of an individual with paternal UPD 16 and a clinically significant phenotype, as well as the first report of an individual with CDG Ia due to UPD. The apparent lack of clinical findings beyond the CDG phenotype supports the hypothesis that there are no areas of imprinting on human chromosome 16.

Global distribution of human progesterone receptor genetic variants. *L. C. Rockwell[1,2], K. Arnson[1], E. J. Rowe[1], F. Jackson[3], J. G. Lorenz[2,4]* 1) Temple University, Philadelphia, PA, 19122; 2) Central Washington University, Ellensburg, WA, 98926; 3) University of Maryland, College Park, MD, 20742; 4) Coriell Institute for Medical Research, Camden, NJ, 08103.

The progesterone receptor (PR) is a transcription factor that mediates the physiological actions of progesterone. Genetic variants of the PR gene have been investigated for associations with reproductive cancers (breast, ovarian, endometrial and prostrate) as well as other disorders and phenotypes (uterine fibroids, recurrent abortion, implantation failure following IVF, panic disorder, prolactin levels, and mammographic density) in several study populations self-identified as white/Caucasian. Little information exists about the frequency of potentially risk-conferring or protective variants in non-white populations. We genotyped 289 individuals from 21 populations derived from Europe, Asia, Africa, the Middle East, and North America, for 4 genetic variants of the PR (+331 G/A, PROGINS, rs561650, and rs608995). These populations include ethnic groups, geographically defined groups, and CEPH samples from Utah. Samples were obtained from Coriell Institute (Camden, NJ) or collected in the field with informed consent. +331 G/A, a single-base pair substitution in the promoter region which may increase transcription of the B isoform of the progesterone receptor protein, is polymorphic in all European populations but not elsewhere with the exception of one population in the Middle East (Druze) and one in Asia (Adygei). PROGINS, which includes an Alu insertion in intron G, occurs at highest frequency in the Middle East and European populations, lower frequency in the African-American population and CEPH samples, and is completely absent in the African and Asian populations excepting the Adygei. SNP rs561650 is polymorphic is all major geographic regions but absent in three populations: Senegal, Iberia and Cambodia. SNP rs608995 is polymorphic in all major geographic regions and all populations studied. The prevalence of progesterone-related reproductive cancers and disorders may differ among populations in part due to variable frequencies of these polymorphisms. (Support from Temple University.).

Genome-wide association study of vitamin D levels in Hispanic Americans: the IRAS Family Study. *C. D. Engelman[1], C. D. Langefeld[2], J. Ziegler[2], K. Taylor[3], J. I. Rotter[3], T. E. Fingerlin[4], J. M. Norris[4]* 1) University of Wisconsin, Madison, WI; 2) Wake Forest University, Winston-Salem, NC; 3) Cedars-Sinai Medical Center, Los Angeles, CA; 4) University of Colorado, Denver, CO.

Purpose: Vitamin D deficiency is associated with many adverse health outcomes, including several bone diseases, more than a dozen types of cancer, multiple autoimmune diseases, and type 2 diabetes. Two metabolites of vitamin D are commonly measured in blood, 25-hydroxyvitamin D (25[OH]D) and 1,25-dihydroxyvitamin D ($1,25[OH]_2D$). A few candidate gene association analyses and one linkage analysis of these vitamin D metabolites have been reported, however this is the first reported genome-wide association study. **Methods**: As part of the Insulin Resistance Atherosclerosis (IRAS) Family Study, 229 Hispanic Americans from 34 families in San Antonio, Texas were genotyped using the Illumina HumanHap300 BeadChip. Assuming an additive genetic model, SNPs were individually tested for association with 25[OH]D and $1,25[OH]_2D$ using variance components analysis implemented in SOLAR, adjusting for age, sex, body mass index, season of blood draw, and admixture for 25[OH]D and age, sex, 25[OH]D, and admixture for $1,25[OH]_2D$. **Results**: Of the 300,139 SNPs meeting all quality control criteria, three were significantly associated with 25[OH]D (p-value$5x10^{-6}$), two with unknown function on chromosome 1 (r^2=0.89) and the third on chromosome 9. Six SNPs were associated with $1,25[OH]_2D$ (p-value$5x10^{-6}$), four of which are all in the *DAB1* gene on chromosome 1 (r^2>0.58). The strongest overall signal was for an intronic SNP on chromosome 1 in *DAB1* (rs6680429) in association with 1,25[OH]2D (p-value=$6.63x10^{-9}$). **Conclusion**: We identified strong statistical evidence for three new SNPs associated with 25[OH]D and six new SNPs associated with $1,25[OH]_2D$ in an Hispanic American population that need to be further studied and replicated. These findings are important because 25[OH]D and $1,25[OH]_2D$ have a strong genetic component and serve as endophenotypes or biomarkers for multiple health outcomes. This study is also significant because Hispanics have been underrepresented in vitamin D research.

Evolutionary Forces Shape the Human RFPL1,2,3 Genes toward a Role in Neocortex Development. *S. I. Nikolaev[1], J. Bonnefont[1], A. L. Perrier[2], S. Guo[3], L. Cartier[1], S. Sorce[1], T. Laforge[1], L. Aubry[2], Ph. Khaitovich[3], M. Peschanski[2], S. E. Antonarakis[1], K.-H. Krause[1]* 1) Dept Genetic Med, Development, Univ Geneva, Geneva, Switzerland; 2) I-STEM, INSERM/UEVE UMR 861, Genopole Campus 1, 5 rue Henri Desbruères, 91030 Evry, France; 3) Institute for Computational Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, 320 Yue Yang Road, 200031 Shanghai, China.

The size and organization of the brain neocortex dramatically changed during primate evolution. This is likely due to the emergence of novel genes after duplication events, evolutionary changes in gene expression, and/or acceleration in protein evolution. Here we describe a human Ret Finger Protein-Like (hRFPL)1,2,3 gene cluster on chromosome 22, which is transactivated by the corticogenic transcription factor Pax6. High hRFPL1,2,3 transcript levels were detected at the onset of neurogenesis in differentiating human embryonic stem cells and in the developing human neocortex, while the unique murine RFPL gene is expressed in liver but not in neural tissue. The history of the RFPL gene family revealed that the RFPL1,2,3 gene ancestor emerged after the Euarchonta-Glires split. Then, duplication events led to the apparition of multiple RFPL1,2,3 genes in Catarrhini (~34 mya), with an emphasis in the hominoid lineage as only human and orangutan kept the functionality of the three genes. In Catarrhini, RFPL1,2,3 expression diverged toward the neocortex and cerebellum over the liver. Importantly, humans showed a striking increase in cortical RFPL1,2,3 expression in comparison to their cerebellum and to chimpanzee and macaque neocortex. Acceleration in RFPL protein evolution was also observed with signs of positive selection in the RFPL1,2,3 cluster after the duplication events and two neofunctionalization events (acquisition of a specific RFPL-Defining Motif in all RFPLs and of a N-terminal 29 amino-acid sequence in catarrhinian RFPL1,2,3). Thus, we propose that the recent emergence and multiplication of RFPL1,2,3 genes contribute to changes in primate neocortex size and/or organization.

Hox genes are associated with clubfoot. *A. R. Ester[1], A. Burt[2], A. Scott[3], C. A. Gurnett[4], M. B. Dobbs[4,5], S. H. Blanton[2], J. T. Hecht[1,3]* 1) Univ Texas Medical School, Houston, TX; 2) Univ of Miami, FL; 3) Shriners Hospital for Children, Houston, TX; 4) Washington School of Medicine, St. Louis, MO; 5) Shriners Hospital for Children, St. Louis, MO.

Clubfoot is a common birth defect occurring in 1/700-1000 live births, and is a complex disease, with the environment, genetic variation and genetic-environmental interactions contributing to this birth defect. *HoxA* and *HoxD* gene clusters are involved in axial and limb patterning during embryonic development, and mutations in both *HoxA* and *HoxD* cause limb defects and syndromes with limb malformations. Because these genes regulate limb development, this study investigated the possibility that variations in *HoxA* and *HoxD* genes play a role in clubfoot. Twenty SNPs spanning these genes were genotyped in 160 nonHispanic white and 213 Hispanic trios and 121 nonHispanic white and 93 Hispanic families. A separate clubfoot population of 144 nonHispanic white trios was used in confirmation studies. Two SNPs in the 3 region of the *HoxD* gene cluster showed minimally significant altered transmission (p=0.02, p=0.04), but the closest *HoxD* genes are not expressed in the limb. Three SNPs in the *HoxA* cluster were significantly over-transmitted, and one of these was significantly over-transmitted in both the primary (p=0.0015) and secondary populations (p=0.028). This SNP is located in the basal promoter of *HoxA9* which is expressed during limb development. Gene-gene interactions were identified between variations in *HoxA* and *HoxD* with variations in apoptotic genes (*Apaf1*, *Bid*, *Casp3*, *Casp9* and *IGFBP3*) that were previously found to be associated with clubfoot. Interestingly, the most significant interactions were found between *Casp3* and *Bid* with both *HoxA* and *HoxD* variants. These interactions suggest a biologic model for clubfoot, with both *Hox* and apoptotic genes acting in a common mechanistic pathway. Interestingly, the SNP in the *HoxA9* promoter is 4kb downstream of a microRNA, miR196-b, which regulates both apoptotic and *Hox* genes. We have identified a previously unreported SNP that is two bases upstream of miR196-b. Together, this genetic variation may contribute to clubfoot.

Accounting for physical activity when testing for association of quantitative phenotypes with GWAS-derived candidate genes for diabetes: The IRAS Family Study. *S. Sibbel[1], J. Ziegler[2], N. Palmer[2], K. Young[1], D. Bowden[2], T. Fingerlin[1], R. Bergman[3], J. Norris[1]* 1) Colorado School of Public Health, Denver, CO; 2) Wake Forest University School of Medicine, Winston-Salem, NC; 3) Keck School of Medicine, University of Southern California, Los Angeles, CA.

Purpose: GWAS have identified several polymorphisms for type 2 diabetes. We recently published associations of these polymorphisms with 3 quantitative measures of diabetes phenotypes in the IRAS Family Study: insulin sensitivity (SI), acute insulin response (AIR), and disposition index (DI)(Palmer et al, 2008). Here, we tested for potential modifying effects of physical activity on these associations. Methods: Energy expenditure (EE) was collected at the time of the frequently-sampled IV glucose tolerance test, using a 1-year physical activity recall, in 989 Hispanic and 468 African-American non-diabetic participants from 134 families. We selected 13 SNPs in HHEX, SLC30A8, EXT2, IGFBP2, CDKAL1, FLJ39370, LOC387761, and an intragenic locus previously associated with our phenotypes for evaluation with EE. In SOLAR, we used a 2 df genotypic test to evaluate each SNP with and without adjusting for EE, to examine if EE explained variation in the phenotype, and whether accounting for EE modified the SNP association. We then tested for an interaction between EE and each SNP on our phenotypes to examine whether the genotypic association differed by levels of physical activity. Results: Adjusting for age, sex, body mass index, site and SNP, lower EE was significantly associated with lower SI and DI but not with AIR ($p<0.006$, <0.03, and $>>0.05$, respectively) in Hispanics. However, adjustment for EE did not appreciably alter the p-value of the SNP associations with SI, DI nor AIR. We also found no significant interactions between the SNPs and EE on SI, DI, nor AIR. Similar findings were observed in the African-American sample. Conclusions: In this example, accounting for physical activity did not alter the associations of these SNPs with SI, DI nor AIR. Regardless, we posit that in complex genetic traits, gene-environment interactions exist and formal testing of hypotheses of such interactions are critical next steps.

Identification of Seven Novel EIF2B Mutations in Chinese Patients with Vanishing White Matter Disease. *Y. Jiang, J. Wang, Y. Wu, Y. Yang, L. Du, J. Qin, X. Wu* Pediatrics, Peking Univ First Hospital, Beijing, Beijing, China.

[Purpose] Vanishing White Matter disease (VWM)is an AR transmitted leukoencephalopathy with specific abnormality in cranial MRI-almost all cerebral and partial cerebellar white matter became demyelination with partial replacement by cerebrospinal fluid (CSF). In 2001-2002, VWM was found to be caused by mutation in any of the five genes (EIF2B1, EIF2B2, EIF2B3, EIF2B4, EIF2B5) which encoding the subunits of eukaryotic translation initiation factor eIF2B (eIF2B~). This is the first hereditary human disease due to the direct defects in protein translation process. [Methods] Informed consents were obtained from all the families involved. Genomic DNA was extracted from peripheral leukocytes. Mutation screening for coding area and flanking introns of EIF2B5, EIF2B4, EIF2B2, EIF2B3 and EIF2B1 were performed in each patient sequential until mutation had been found. For novel mutations, the corresponding amplicons from fifty control samples were analyzed. [Results] Mutations were identified in 9 patients out of 11 patients with clinical diagnosis of VWM, including 5 patients with EIF2B5 mutations and the other 4 patients with EIF2B3 mutations, no EIF2B4, EIF2B2, and EIF2B1 mutation found. Totally 12 kinds of mutations were found, including 5 novel missense mutations: c.1126 A>G (p.N376D), c.1004 G>C (p.C335S), c.185 A>T (p.D62V), c.140 G>A (p.G47E), c.1037 T>C (p.I346T). 5 reported missense mutations: c.943 C>T (p.R315C), c.1340 C>T (p.S447L), c.674 G>A (p.R225Q), c.1016 G>C (p.R339P), c.1157 G>C (p.G386V), one novel nonsense mutation: c.805 C>T (p.R269X), one novel deletion mutation: c.1827_1838del (p.S610_D613del). Mutations of six patients had been proved inherited from their parents. [Conclusion] We identified 7 novel mutations in Chinese VWM patients. This is the first report of VWM in Chinese. 58% (7/12) mutations were novel mutations, and 44% (4/9) patients with EIF2B3 (much higher ratio than reported). This indicated that the mutation spectrum should be different in Chinese from other ethnic groups. The functional analysis of these novel mutations is ongoing now.

Clinical, neuropathological and genetic studies of Chinese patients with infantile neuroaxonal dystrophy. *Y. Wu[1], Y. Jiang[1], Z. Gao[1], J. Wang[1], Y. Yuan[2], H. Xiong[1], X. Chang[1], X. Bao[1], Y. Zhang[1], J. Xiao[3], X. Wu[1]* 1) Department of Pediatrics, Peking University First Hospital, Beijing, China; 2) Department of Neurology, Peking University First Hospital, Beijing, China; 3) Department of Radiology, Peking University First Hospital, Beijing, China.

Infantile neuroaxonal dystrophy (INAD) is a rare autosomal recessive neurodegenerative disorder.The clinical picture is characterized by progressive psychomotor regression with onset between 6 months to 2 years of age, usually leading to death by 10 years. The presence of axonal swelling is the most typical neuropathological finding. Before the identification of the mutations in PLA2G6 encoding iPLA2-VIA (cytosolic Ca2+-independent phospholipids A2, group VIA) in 2006, the neuropathological data was critical for definitive diagnosis. Only three genetic studies on INAD patients were published worldwide, with 44 mutations identified .Only two patients confirmed by neuropathology have been reported in China up till now. **Purpose** To delineate clinical, pathological and genetic characteristics of Chinese patients with INAD, we analyze ten cases. **Methods** Extensive clinical investigations were performed in ten patients with clinical diagnosis of INAD, as well as neuropathological analysis. All patients were screened for mutations in PLA2G6. **Results** All cases showed the typical clinical features of INAD. The presence of axonal swelling ,which is a hallmark of pathological finding in INAD,was found in biopsy specimens from three cases. 12 different PLA2G6 mutations were identified, consisted of 9 novel and 3 previously reported mutations. The 9 novel mutations include 6 missense mutations (R39Q, V371M, G373R, K545E, R591W and A657V), 1 abolishing the normal start codon (c.1A>G), 1 nonsense mutation (R70X) and 1 splice site mutation (IVS10+1G>A). **Conclusions** The clinical features of Chinese patients with INAD are consistent with those described in previous reports on patients from other races. 9 novel mutations identified in this study greatly broadened the spectrum of PLA2G6 mutations. In addition to pathological evidence, genetic analysis is an useful tool for definitive diagnosis of INAD.

Insulin-like Growth Factor Binding Protein-3 is associated with clubfoot. *K. S. Weymouth[1,2], A. R. Ester[1,2], A. Burt[3], S. H. Blanton[3], J. T. Hecht[1,2]* 1) University of Texas Medical School, Houston, TX; 2) Graduate School of Biomedical Sciences, Houston, TX; 3) University of Miami Miller School of Medicine, Miami, FL.

Clubfoot, a common birth defect affecting 1/1000 livebirths, is characterized by equinus of the ankle, varus of the hindfoot and adductus of the forefoot. Because both genetic and environmental factors have been causally implicated, a multifactorial etiology has been suggested for this complex disorder. In previous studies, we have shown that *HoxA* and *D* and mitochondrial-mediated apoptotic pathway genes are associated with clubfoot. Insulin-like growth factor binding protein-3 (*IGFBP3*) is of interest because it has a proapoptotic role in certain cancers and is underexpressed in mutant *HOXA13* mice. Because apoptosis and HOX genes play critical roles in limb development and were recently found to be associated with clubfoot, we hypothesized that *IGFBP3* may also have a causal role in clubfoot. This study was undertaken to investigate whether variation in *IGFBP3* contributes to clubfoot. Twelve SNPs spanning *IGFBP3* were genotyped in 160 nonHispanic white and 213 Hispanic simplex trios and 121 nonHispanic white and 93 Hispanic multiplex families. The data was analyzed in aggregate and, because allele frequencies were different, the data was stratified by ethnicity (nonHispanic white and Hispanic). In the combined sample, 5 SNPs had significantly altered transmission (0.007p-value0.05). The stratified data identified 4 significantly associated SNPs (rs3793345, p=0.0004) in the Hispanic population. Pairwise haplotype analysis detected significant overtransmission for 25 haplotypes with the strongest haplotype association identified for rs13223993 and rs3793345 (p=0.002). Gene-gene interaction was detected between SNPs in *IGFBP3* and *HOXA*, *HOXD* and mitochondrial-mediated apoptotic genes. These results suggest that genetic variation in *IGFBP3* plays a role in clubfoot and interacts with other genetic variants associated with clubfoot. It also provides the first evidence of gene-gene interaction and a common pathway leading to clubfoot.

A comparison of models used to predict *MLH1, MSH2* and *MSH6* mutation carriers. *C. J. Pouchet[1], M. Tischkowitz[2,3], N. Wong[1,4], W. Foulkes[2,3], G. Schneider[5], B. Rosen-Sheidley[5]* 1) Department of Human Genetics, SMBD-Jewish General Hospital, Montreal, Quebec, Canada; 2) Department of SMBD-Jewish General Hospital, Human Genetics, Oncology and Medicine, Montreal, Quebec, Canada; 3) Faculty of Medicine, McGill University, Montreal, Quebec, Canada; 4) Department of Human Genetics, McGill University, Montreal, Quebec, Canada; 5) Genetic Counseling Program, Brandeis University, Waltham, MA.

BACKGROUND: MMRpro, PREMM$_{1,2}$ and MMRpredict are three models which have been developed to predict the probability that an individual carries a Lynch syndrome-causing mutation. Each model utilizes data from both personal and family histories of cancer. To date, no studies have compared the performance of these models in a cancer genetics clinic. The purpose of this study was to determine each models ability to predict the probability of carrying a germline mismatch repair gene mutation in individuals with a family history of colorectal cancer, and to determine the clinical applicability of the models. METHODS: We obtained family pedigrees from 81 individuals who presented for HNPCC testing due to a personal and/or family history of cancer. Data from each pedigree were entered into MMRpredict, PREMM$_{1,2}$ and MMRpro, and were analyzed using SPSS. RESULTS: We found that MMRpredict, PREMM$_{1,2}$ and MMRpro showed similar performances with areas under the ROC curve of 0.731, 0.765 and 0.732 respectively. PREMM$_{1,2}$ showed the least dispersion of mutation probability estimates compared to the other two models with a p-value of 0.205, compared to 0.034 for MMRpro and 0.001 for MMRpredict. CONCLUSION: We found all three performed well in a cancer genetics setting, with PREMM$_{1,2}$ giving slightly better estimates. However, there were some significant discrepancies between the models in cases where the proband had both endometrial and colorectal cancer.

Evaluation of the QIAsymphony system with genetic research applications. *K. Beller[1], C. Homberg[1], S. Hammerschmidt[1], C. Kiss[1], P. Gohl[2], G. Wildhardt[3], K.-U. Lentes[3], C. Lenz[1]* 1) R&D Department, QIAGEN GmbH, Hilden, Germany; 2) Bioscientia, Institute for Medical Diagnostics, Ingelheim, Germany; 3) Bioscientia, Center for Human Genetics, Ingelheim, Germany.

Introduction: Blood is the most common sample material for genetic testing. In this study we determined if yield and quality of DNA purified from blood with the QIAsymphony system is suitable for use in genetic testing methods. Material and Methods: DNA was isolated from 200 l and 1000 l human whole blood in primary tubes with QIAsymphony DNA Mini or Midi Kits. DNA yield and quality were determined spectrophotometrically and by agarose gel electrophoresis. Performance of the purified DNA was tested with a set of 15 different genetic tests. Results: Yields of total DNA ranged from 3.9 - 12.8 g and 18.5 - 45.1 g from 200 and 1000 l blood, respectively. The DNA showed high integrity and was highly pure, with a mean A260/A280 ratio of 1.85 0.06. The DNA performed well in MLPA analyses (CAH and DMD) as well as with a commercially available cystic fibrosis assay, indicated by distinct and accurate electropherogramm profiles. SNP diagnostics by either real time PCR with melting curve analysis or pyrosequencing resulted in correct genotyping of Factor V, Factor II, HFE, MTHFR, LCT, PAI-1 and DPD alleles. HLA typing of loci A, B, C and DR for several individuals was successful. DNA purified with the 1000 l blood protocol was well suited for Fragile-X syndrome analyses by Southern blot. Conclusion: The QIAsymphony system enables fully automated purification of total genomic DNA of up to 96 human whole blood samples from primary blood tubes. Purified DNA shows high quality and is well suited for use in a broad range of genetic testing procedures. Disclaimer: The QIAsymphony SP is intended to be used only in combination with QIAsymphony kits indicated for use with the QIAsymphony SP for applications described in the respective QIAsymphony kit handbooks. The QIAsymphony DNA kits are intended for general laboratory use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

Interim results of a Phase II, multicenter, open-label study of sapropterin dihydrochloride in subjects with hyperphenylalaninemia related to primary BH_4 deficiency. *M. Wasserstein[1], B. Burton[2], S. Cederbaum[3], J. Munser[4], R. Scott[5], C. Harding[6], U. Wendel[7], C. Whitley[8], J. Wolf[9], A. Dorenbaum[10], E. Foehr[10], E. Kakkis[10]* 1) Mount Sinai Sch of Med, New York, NY; 2) Children's Memorial Hosp, Chicago, IL; 3) UCLA Med Ctr, Los Angeles, CA; 4) U N Carolina, Chapel Hill, NC; 5) U Washington, Seattle, WA; 6) Oregon Health & Sci Uni, Portland, OR; 7) Uni Childrens Hosp, Dusseldorf, Germany; 8) U Minnesota, Minneapolis, MN; 9) U Wisconsin, Madison, WI; 10) BioMarin Pharmaceutical Inc., Novato, CA.

We evaluated the safety and efficacy of sapropterin dihydrochloride (sapropterin) to manage blood Phe in subjects with primary BH_4-deficiency. Of the 12 subjects (5M/7F; mean age 13.59.6 [range: 3-35] years) with a history of Phe 180 mol/L, 6 had 6-pyruvoyl-tetrahydropterin synthetase, 3 had dihydropteridine reductase (DHPR), and 3 had unidentified BH_4 synthesis defects. All had normal ALT levels, were not taking investigational agents, and were not pregnant or breastfeeding. Subjects receiving non-registered BH_4 continued pre-study treatment for 2 wks. At Wk 3, they switched to sapropterin at the same dose and subjects not previously receiving BH_4 began sapropterin 5 mg/kg/day divided bid. Dose could be adjusted up to 20 mg/kg/day. Diet was unchanged. All subjects with BH_4-production defects and none with recycling defects were pre-treated with non-registered BH_4. MeanSD non-registered BH_4 dose at enrollment was 4.12.5 mg/kg/day. At Wk 10, 9 subjects received sapropterin 5 mg/kg/day and 3 received 10 mg/kg/day. Before sapropterin, mean blood Phe was 72.213.4 and 315.0180.9 mol/L for the BH_4 production- and recycling-defect groups, respectively; at Wk 10 on sapropterin, mean blood Phe was 75.011.5 and 347.7187.7 mol/L, respectively. Sapropterin was well tolerated. There were no deaths, serious adverse events (SAEs) or withdrawals caused by the drug; 9 subjects had AEs. Most frequently reported AEs were diarrhea (N=4) and vomiting (N=3). Dystonia unrelated to drug (N=1) was the only AE considered severe. Data from this interim report support a favorable profile for sapropterin in subjects with primary BH_4 deficiency caused by loss of a BH_4-production enzyme such as DHPR.

Analysis of the breakpoint junctions in six subjects with terminal deletions of 1p36. *C. S. D'Angelo[1], M. Gajecka[2], L. G. Shaffer[2,3], C. P. Koiffmann[1]* 1) Depto Genética, Universidade de São Paulo, SP, Brazil; 2) Washington State University, Spokane, WA, USA; 3) Signature Genomic Laboratories, Spokane, WA, USA.

Molecular characterization of subjects with monosomy 1p36 indicates these deletions have non-recurrent breakpoints; resulting in terminal truncations, interstitial deletions, complex rearrangements, and derivative chromosomes. In addition, cryptic interrupted inverted duplications have been observed at the end of terminally deleted chromosomes. Premeiotic breakage-fusion-bridge cycles appear to be an intermediate step in the process of generating and stabilizing these terminal deletions. Stabilization by telomere healing has been confirmed in one pure terminal truncation of 1p36. Telomeric acquisition from another chromosome most likely occurs by break-induced replication and nonhomologous end joining, appears to play a role in most rearrangements. Thus, the mechanisms are complex and no one mechanism is responsible for all rearrangements of this region. Six rearrangements of 1p36 were characterized. These included two apparently pure terminal truncations, three derivative chromosomes, and a complex rearrangement. Microarray analysis revealed a terminal deletion of ~2 Mb followed by a ~300 kb copy-number gain. Breakpoint junction analysis uncovered a cryptic interrupted inverted duplication at the end of the deleted chromosome. Array CGH and FISH analysis of unrelated der(1)t(1;1)(p36;q44) chromosomes from two subjects revealed variability at the breakpoint locations. In one case, no evidence for substantial homology between the 1p and the 1q breakpoints was observed. Preliminary data for the other case indicate the 1q breakpoint falls within a genomic gap. Sequence analysis of a der(1)t(1;4)(p36;q35) did not show any sequence homology at the 1p and 4q junctions. Characterization of two additional apparently pure terminal truncations narrowed the breakpoints to genomic intervals ~3 kb long containing a series of 1p36 specific segmental duplications. Collectively, these cases represent the complexity observed for most rearrangements of 1p36 and indicate that a variety of mechanisms result in the breakage and stabilization of these chromosome abnormalities. CEPID-FAPESP, CNPq.

Detection of variations in the p53 gene using True Single Molecule Sequencing™ Technology. *H. Gao[1], J. I. Colonell[2], C. Hart[2], D. Lipson[2], J. Sram[1], C. Fong[1], J.-S. Saldivar[1], P. M. Milos[2], W. E. Pierceall[2], J. Reifenberger[2], J. F. Thompson[2]* 1) City of Hope National Medical Center, Duarte, CA 91010, USA; 2) Helicos BioSciences, Cambridge, MA 02139, USA.

Somatic changes in DNA have been shown to play a large role in the initiation and development of cancer. Because these changes can occur throughout the course of tumorigenesis and metastasis, it is important to be able to detect novel variations once they occur to couple with an appropriate diagnosis. DNA samples for such analysis are now routinely collected via micro-dissection to remove the normal tissue or select particular tumor cells for tumor somatic mutation analysis. To address this issue, we sequenced individual and mixed DNA samples using True Single Molecule Sequencing (tSMS)TM technology in which individual templates of DNA were analyzed using a proprietary form of sequencing-by-synthesis. P53 was used as a model gene to investigate DNA samples from many patients with documented variations and insertions/deletions in the gene obtained through traditional Sanger sequencing. Bioinformatic tools were developed to detect SNPs and indels in the short read data produced by Helicos tSMSTM technology. Using these tools we detected variations in individual patient DNA samples, as well as in sample mixtures derived from multiple patients. A major benefit with the tSMSTM approach relies on our ability to directly sequence DNA samples without prior amplification thereby having the potential to eliminate a major source of artifactual results introduced by sample manipulation.

A novel missense mutation in the HAX1 gene and neutrophils apoptosis in severe congenital neutropenia (Kostmann disease) patients. *M. Faiyaz-Ul-Haque[1], A. Al-Jefri[2], H. A. Abalkhail[1], M. Toulimat[1], M. A. Al-Muallimi[1], M. S. Pulicat[3], A. Gaafar[3], A. A. Alaiya[3], F. Al-Dayel[1], S. H. E. Zaidi[4]* 1) Pathology and Laboratory Medicine, King Faisal Specialist Hospital & Research Centre, Riyadh, Saudi Arabia; 2) Pediatric Hematology/Oncology, King Faisal Specialist Hospital & Research Centre, Riyadh, Saudi Arabia; 3) Biological and Medical Research, King Faisal Specialist Hospital & Research Centre, Riyadh, Saudi Arabia; 4) Department of Medicine, University Health Network & University of Toronto, Ontario, Canada.

Autosomal recessive, severe congenital neutropenia (SCN) is caused by maturation arrest of granulopoiesis at the level of promyelocytes. This disorder is produced by mutations in the HAX1 gene. This gene encodes for the HAX1 (HCLS1-associated protein X1) protein which is indispensable for maintaining inner mitochondrial membrane potential and for protection against apoptosis in myeloid cells. SCN patients with HAX1 gene mutations display neutrophils apoptosis in addition to neurological symptoms which are manifested when mutation affects both transcript variants of the HAX1 gene. Here, we describe a consanguineous Saudi Arabian family in which patients were clinically diagnosed with the SCN. DNA sequencing identified a novel missense mutation in the HAX1 gene of these patients. This mutation is present in both transcript variants of the HAX1 gene. Interestingly these patients do not exhibit seizures, epilepsy, developmental delay or any other apparent neurological symptoms. On the other hand, consistent with the SCN phenotype, neutrophils form one of these patients exhibited massive apoptosis as assessed by flow cytometry analysis. This study suggests that the manifestations of neurological phenotype in SCN patients may depend upon the nature of mutation and that a complete absence of HAX1 protein may be required for expression of the neurological symptoms in the SCN patients.

Expression profiling and methylation-specific MLPA in uveal melanoma: markers for tumor progression and survival? *A. deKlein[1,4], H. Mensink[2], W. van Gils[1,3], J. Vaarwater[1,2], E. Kilic[3], D. Paridaens[2]* 1) Clinical Genetics, Erasmus MC, Rotterdam, Netherlands; 2) Ophthalmology, Rotterdam Eye Hospital, Rotterdam, Netherlands; 3) Ophthalmology, Erasmus MC, Rotterdam, Netherlands; 4) ROTTERDAM OCULAR MELANOMA STUDYGROUP.

Introduction: Uveal melanoma (UM) is the most common primary intra-ocular malignant tumour. Although chromosomal aberrations have prognostic significance, they result in classification errors in prediction of patient survival. Gene-expression profiling was performed and revealed sensitive prognostic markers and relevant genes in the carcinogenesis of UM. Besides genetic factors, epigenetic events, such as promoter silencing by methylation, play an important role in tumour progression. **Materials and Methods:** For expression profiling we analysed 49 tumour RNAs on Affymetrix GeneChips. Analyses was done with Omniviz and PAM software and validated with real-time PCR. Methylation of specific CpG islands or promoter regions was investigated using a methylation specific MLPA kit in 86 UM samples. **Results:** UM expression profiles classify in two distinct tumour classes with strong prognostic significance ($p<0.001$; hazard ratio 7.7). Using a locally adaptive statistical procedure (LAP) two regions on chromosome-arm 3p with decreased gene expression in tumours with shorter disease-free survival were identified. Promoter methylation of TIMP3, VHL, CDKN2A, CDKN2B and MLH1 was observed in several uveal melanomas and analysis of these data (e.g. correlation with patient survival) is in progress. **Conclusion:** Micro-array classification outperforms all known prognostic indicators for uveal melanomas. Methylation of promoter regions of genes in regions with decreased expression indicates a role for methylation in the aetiology of uveal melanoma.

Analysis of copy number variation in sporadic autism. *K. J. Meyer[1], L. K. Davis[1], D. S. Rudd[1], E. M. Stone[2,4], V. C. Sheffield[3,4], T. H. Wassink[1]* 1) Department of Psychiatry, University of Iowa, Iowa City, IA; 2) Department of Ophthalmology and Visual Sciences, University of Iowa, Iowa City, IA; 3) Department of Pediatrics, University of Iowa, Iowa City, IA; 4) Howard Hughes Medical Institute, University of Iowa, Iowa City, IA.

Autism is a pervasive developmental disorder characterized by impairments in communication and social interaction as well as restricted interests and repetitive behaviors. Previous data has indicated de novo copy number variants (CNVs) are enriched in sporadic cases of autism. Therefore, we screened the DNA of 38 patients with sporadic autism and their parents (when available) for CNVs using the Affymetrix 250K SNP microarray. We identified two individuals with CNVs on chromosome 16p11.2, two individuals with duplications of chromosome 15q11.2-q13.1, two individuals with deletions on 2p16.3 in the NRXN1 gene, and two individuals with CNVs on chromosome 22q11.21 in the DiGeorge Syndrome region. Together, these CNVs account for 21% of sporadic autism in our sample. In addition, we identified multiple de novo and novel CNVs that may play a role in the pathogenesis of autism.

Socio-economic and spatial population structure in Northern Finland. *C. Hoggart[1], L. Coin[1], L. Peltonen[2], M. McCarthy[3], N. Freimer[4], P. Elliott[1], D. Balding[1], M. Järvelin[1]* 1) Epidemiology & Public Health, Imperial Col, London, UK; 2) Wellcome Trust Sanger Institute, Cambridge, UK; 3) Oxford Centre for Diabetes, Endocrinology and Metabolism, Oxford, UK; 4) Semel Institute for Neuroscience and Human Behavior, Los Angeles, CA.

Principal component (PC) analysis of genome-wide genotype data is widely used to summarise population structure. PCs from genotype data from the Northern Finnish Birth Cohort (NFBC), based on 4,763 individuals and 61,917 SNPs sampled from the Illumina 370K chip, show striking association with spatial location. In particular, the genetic variation described by the first two PCs is consistent with an east-west cline of genetic variation and with distinct early and late settlement patterns, as previously reported. However, the residuals of PCs after regression on latitude and longitude show association with socio-economic status (SES), which indicates patterns of assortative mating according to SES over many generations in a manner that cannot be captured by simple linear directional population gradients. The SES of each individuals parents was recorded in one of six categories, including two classifications for farmers (> 8 ha, and <= 8 ha). After adjustment for longitude and latitude, four of the first 10 PCs were significantly associated with mothers SES ($p < 10^{-7}$); and three with fathers SES. The PCs exhibited greatest variation between the large farmers, small farmers and professionals. A predictive model for SES using the PCs was able to predict 50% of individuals whose mothers were professionals, 49% of individuals whose mother or father were small farmers and 48% of individuals whose fathers were large farmers, at a false positive rate of 20%. Finally we explored the effect of population structure in the NFBC on the genome-wide association analysis (GWAS) of four phenotypes: height, LDL, total cholesterol and pulse pressure. All phenotypes showed significant associations with location, SES and the PCs; however, these associations could be adequately accounted for in these GWAS studies using PCs alone.

Organization of the 21-II region of human chromosome 21. *W. Ziccardi, R. Ennesser, M. Bozovsky, C. Zhao, J. Doering* Dept. of Biology, Loyola University Chicago.

The human genome sequence does not include the heterochromatic regions, although these sequences comprise 10-15% of the genome. We are constructing a detailed physical map of the HC21 centromere and short arm as a model for the organization of these regions. Our previous work showed that the alphoid DNA present on HC21 is found in two distinct regions: D21Z1 and 21-II. The D21Z1 cluster, required for centromere function, is 1 Mb long and consists of a homogeneous array of 11-mer higher order repeats (HOR). This alphoid array does contain substantial sequence heterogeneity at both its ends. We have now determined that the 21-II region on the p arm is comprised of five separate, distinct clusters (Mp1-Mp5) of monomeric -satellite repeats that extend at least 3.0 Mb away from D21Z1. Centromere-associated monomeric alphoid sequences extending this far from the centromere have not previously been described on other chromosomes. Interspersed between these monomeric alphoid clusters are satellite III clusters, multiple copies of the chAB4 duplicon, and other low copy number repeats that have not yet been fully characterized. The sizes for the Mp1-Mp5 clusters are highly heterogeneous, with size estimates of at least 94 kb, 25 kb, 126 kb, 30 kb and 50 kb, respectively. The respective distances of the clusters from the distal end of D21Z1 are approximately 1.0 Mb, 1.4 Mb, 1.7 Mb, 2.3 Mb, and at least 3.0 Mb. Detailed representative sequence information has been obtained for each of the 21-II alphoid clusters using 21p BACs already in GenBank as well as new clones we acquired. Analysis of this alphoid DNA shows no evidence of any of the regular repeating restriction sites or CENP-B boxes characteristic of higher-order alphoid repeats. Dot-plot analysis further indicates that this is monomeric alphoid DNA. These sequences show only 70-80% sequence identity to D21Z1 and to each other. The monomeric alphoid sequences form evolutionary clades distinct from D21Z1. Such multiple monomeric alphoid clusters are consistent with models suggesting that the functional centromere may move along the chromosome over evolutionary time.

Association of COMT Val158Met with autism spectrum disorders in Thai children. *P. Limprasert[1], W. Maisrikaw[1], T. Sripo[1], N. Ruangdaraganon[2], T. Hansakunachai[3], R. Roongpraiwan[2], T. Sombuntham[2], J. Cui[4], X. Guo[4]*
1) Human Genetics/Dept Pathology, Prince of Songkla Univ, Songkhla, Thailand; 2) Dept Pediatrics, Ramathibodi Hospital, Mahidol Univ, Bangkok, Thailand; 3) Dept Pediatrics, Fac Medicine, Thammasat Univ, Pathumthani, Thailand; 4) Cedars-Sinai Medical Center, Los Angeles, CA, USA.

Catechol-*O*-methyl-transferase (*COMT*) gene is located on chromosome 22q11.2. COMT enzyme catalyzes the inactivation of catecholamines including adrenaline, noradrenaline and dopamine. The Val (G allele) of SNP Val158Met at codon 158 of the *COMT* gene leads to higher COMT activity compared with Met (A allele). Exiting data suggest the involvement of *COMT* Val158Met with several psychiatric diseases including obsessive-compulsive disorder (OCD). Autism spectrum disorders (ASD) are complex neuropsychiatric disorders characterized by limited or absent verbal and nonverbal communication, a lack of reciprocal social interaction, and restricted interest and repetitive behaviors (OCD like symptoms). We investigate here the association between *COMT* Val158Met and Thai ASD children. One-hundred-and-fifty-one patients fulfilling the DSM-IV criteria for autistic disorder (128 Males, 23 females, mean age ~4 years) and 212 normal controls were recruited from two university hospitals in Bangkok. Parents of 130 out of the 151 patients were also recruited into this study. The *COMT* Val158Met polymorphisms were genotyped using allele specific PCR. Association analyses were carried out using PLINK. Significant association was first found under an additive genetic model ($P = 0.04$) in the case-control samples, and was further confirmed in the 130 family trios using the transmission disequilibrium test (TDT). The A allele was more common in patients (37%) than in controls (30%). The TDT test demonstrated a preferential transmission of the A allele to the affected children ($P = 0.04$). Our findings suggest that *COMT* Val158Met plays a role in the genetic predisposition to ASD in Thai children.

22q13.3 deletion syndrome: clinical and molecular analysis using array CGH. *S. U. Dhar[1], D. Del Gaudio[1], J. R. German[1], S. U. Peters[1], P. I. Bader[2], M. Blazo[1], C. W. Brown[1], B. H. Graham[1], T. A. Grebe[3], S. Lalani[1], D. Miller[4], S. Sparagana[5], J. A.III Phillips[6], A. L. Beaudet[1], S. W. Cheung[1], T. Sahoo[1]* 1) Molecular & Human Genetics, Baylor college of Medicine, Houston, TX; 2) Parkview Cytogenetic Laboratory, Fort Wayne, IN; 3) CHC Phoenix Genetics Program, St.Joseph's Hospital & medical center, Phoenix, AZ; 4) Children's Hospital, Boston, MA; 5) Pediatric Neurology, Texas Scottish Rite Hospital for Children, Dallas, TX; 6) Division of Medical Genetics, Vanderbilt University School of medicine, Nashville, TN.

22q13.3 deletion syndrome is a microdeletion syndrome resulting from loss of terminal segments of varying sizes at 22qter. Current data suggests a lack of correlation between the spectrum and severity of phenotype and the size and nature of the deletion. We carried out detailed clinical and molecular characterization of 12 patients. Developmental delay and speech abnormalities were common to all and comparable to previously reported cases. Hypotonia was recorded in 42% cases compared to over 85% in earlier cases. Array-based comparative genomic hybridization (aCGH) revealed the deletions to vary from 230 kb to 9.5 Mb. Five cases had a similar deletion (4.65 Mb) and might imply the recurrent nature of at least one deletion. Gene content and disruptions within the deleted segments were consistent with the size and included the three terminal genes (SHANK3, RAB1 and ACR) in the smallest deletion and over 50 genes in the largest. In the smallest deletion, a combination of aCGH and quantitative real-time PCR revealed the deletion breakpoint to be within the SHANK3 gene. These data support a crucial role for SHANK3 in the neuropsychiatric abnormalities seen in these patients. SHANK3 haploinsufficiency has been identified in some patients with idiopathic autism. Similar microdeletions, if identified, might elucidate additional genotype-phenotype correlations and highlights the use of aCGH as an important diagnostic tool. We recommend aCGH as a standard diagnostic test for patients with developmental delay, unexplained hypotonia and autism spectrum disorder with or without craniofacial dysmorphism.

Detection of 100 - 500 kb chromosome abnormalities: Utilization of a quantitative whole genome SNP array. *S. Schwartz, L. Henderson, D. Conrad, C. Ober, R. Nicolae, D. Waggoner, C. Tan, A. Murmann* Department of Human Genetics, University of Chicago, Chicago, IL.

The advent of array analysis, to detect small deletions and duplications, has created many new questions within the clinical community including: what array should be used?, how small can the changes be that can routinely be detected?, and what patients should be studied? These are complex questions that are not easily answered. To better understand these issues, we have tested a whole genome SNP array to study a series of phenotypically abnormal patients, some with known abnormalities, others were chromosomally normal. Of the 270 patients studied, 85 abnormalities that were not detected by routine G-banding were seen by array analysis; 35 additional cases were found to have more complex rearrangements than originally thought and could be clarified more precisely. In a recent study to explore the array's efficacy in 69 patients with phenotypic abnormalities and normal chromosomes, 19 patients (27.5%) had apparently clinically significant abnormalities. 29 different abnormalities could be detected, almost 35% of which involved less than 500 kb of DNA. This work has raised significant issues regarding the approach to employ when utilizing arrays and have provided interesting results including: (1) The importance of looking for and detecting abnormalities less than 500 kb, as 35% of the abnormalities detected were between 100 - 500 kb; (2) In 79% of cases specific genes related to the phenotype could be identified; (3) The complexity of abnormalities was greater than expected as 16% of the patients with abnormalities had more than one abnormality; (4) In many cases only one specific gene was involved; (5) 16% of the abnormalities had a carrier parent with an increased recurrence risk, indicating the importance of family studies; (6) The utilization of multiple analysis tools allowed a specificity of 97.6% and a sensitivity of 100% for any abnormality greater than 100 kb; and (7) The preliminary analysis of phenotypic abnormalities in our patients did not indicate that a specific phenotypic group, from those patients referred, was more associated with an abnormal array finding.

Functional characterization of *APOH* promoter and its variants. *S. Suresh[1], F. Y. Demirci[1], A. H. Kao[2], C. M. Kammerer[1], S. Manzi[2], M. I. Kamboh[1]* 1) Dept of Human Genetics, Univ of Pittsburgh, Pittsburgh, PA; 2) Lupus Center of Excellence, Univ of Pittsburgh, Pittsburgh, PA.

Apolipoprotein H (APOH), a.k.a. $_2$-glycoprotein I, is a major autoantigen for antiphospholipid antibodies involved in autoimmune diseases, such as systemic lupus erythematosus (SLE) and antiphospholipid syndrome. Sequence variation in the 5' regulatory element could directly affect gene expression and phenotypic variation. The purpose of this study was to characterize the *APOH* promoter and its variants by in vitro functional experiments and examining their relation with human plasma APOH levels. We examined (1) the individual effects of eight SNPs in the 5' flanking region of *APOH* (~1.4 kb) on luciferase activity in COS-1 cells and their impact on plasma APOH levels in 447 U.S. White females, (2) the DNA-binding properties of *APOH* promoter using electrophoretic mobility shift assay (EMSA) in HepG2 cells, and (3) the effects of serial deletion analysis of *APOH* 5' flanking region in COS-1 cells. Dual-luciferase experiments revealed that the variant alleles of three promoter SNPs (-1219G>A, -643T>C and -32C>A) showed significantly lower luciferase expression (51%, 40% and 37%, respectively) as compared to the wild-type allele. EMSA demonstrated that these three variants specifically bind with protein(s) from HepG2 cell nuclear extracts. In deletion experiments, the maximal promoter activity was observed on a fragment extending from position -325 to +43. Further deletion of sequences down to -65 decreased expression dramatically, suggesting the presence of essential elements between -325 and -65 positions of the *APOH* promoter. Three-site haplotype analysis (-1219G>A, -643T>C, and -32C>A) revealed one haplotype carrying -32A (freq: 0.075) to be significantly associated with decreased plasma APOH levels ($P < 0.001$). Another haplotype harboring the minor allele for -1219A (freq: 0.080) showed a significant albeit less pronounced association with plasma APOH levels ($P = 0.046$). In conclusion, we have characterized the functional promoter of *APOH* and identified functional variants that affect the transcriptional activity of the *APOH* promoter.

Patient with multifocal renal cell carcinoma, pancreatic neuroendocrine tumor and TSC1 Q343X mutation, TSC2 SNP and PTEN c.210-7~3del5. *S. Gustafson, C. Eng* Genomic Medicine Inst, Cleveland Clinic Fndn, Cleveland, OH.

A 29-year-old female with bilateral renal and pancreatic masses was referred for von Hippel-Lindau syndrome, an autosomal dominant disorder caused by VHL mutations, characterized by retinal and CNS hemangioblastomas, renal cell carcinoma (RCC), pheochromocytomas and pancreatic tumors. Abdominal CT confirmed solid renal neoplasms and a lobular pancreatic mass. CNS MRI reported subependymal nodules consistent with tuberous sclerosis complex (TSC). Ophthalmology exam was negative. Genetics evaluation revealed a history of learning delay, one partial seizure during adolescence, and un-biopsied skin lesions. Patient reported a brother with a possible spinal hemangioblastoma. Physical exam identified a 2-3 cm hypomelanotic patch and 8-10 cm shagreen patch on her back. A clinical diagnosis of TSC was made. TSC is an autosomal dominant hamartomatous disorder caused by mutations in TSC1 or TSC2, associated with typical skin findings, brain lesions, epilepsy and renal angiomyolipomas, with 2% progressing to renal malignancy. Radical right nephrectomy and distal pancreatectomy were performed. Pathology reported multiple foci of clear cell RCC, angiomyolipomas, a medullary fibroma and a non-functioning pancreatic neuroendocrine tumor. Concurrent molecular analysis of VHL, TSC1 and TSC2 identified a heterozygous Q343X TSC1 mutation and a heterozygous R1329H TSC2 polymorphism. PTEN analysis was performed as part of our cancer banking protocol and revealed a c.210-7~3del5 variant. VHL analysis was negative. TSC1 Q343X is likely deleterious due to its predicted truncating effects. PTEN c.210-7~3del5 has been classified as deleterious by a clinical lab but it is more likely a low penetrance modifier of cancer predisposition. TSC2 is downstream of the PTEN-AKT signaling pathway. TSC1 complexes with TSC2 to inhibit mTOR and HIF1 transcription. In this signaling context, one can visualize the interaction of the patients TSC1 truncating mutation with the PTEN variant leading to exaggerated mTOR signaling. Thus, a rational therapeutic choice should her clear cell carcinoma become metastatic or locally advanced would be combination mTOR inhibition and anti-angiogenic therapy.

LTBP2 mutation in autosomal recessive microspherophakia with marfanoid features. *M. Abramowicz[1,4], Y. Sznajer[2], F. Roulez[3], J. Desir[4]* 1) Dept Genetics, Hosp Erasme - ULB, Brussels, Belgium; 2) Pediatrics, HUDERF - ULB, Brussels, Belgium; 3) Ophthalmology, HUDERF - ULB, Brussels, Belgium; 4) Laboratory of Medical Genetics, ULB, Brussels, Belgium.

Microspherophakia is a lens malformation encountered in Marfan (MFS) and Weill-Marchesani (WMS) syndromes. We observed a large consanguineous family with three children affected with microspherophakia. The proband had tall stature with an arm span larger than his height, long slender fingers, and a high-arched palate. He did not meet the diagnostic criteria for MFS, nor WMS. No mutation was found in the MFS-associated gene *FBN1* (CMG, University of Gent, Belgium). We mapped the locus by homozygosity to a 12.6 cM region of chromosome 14q2 using a 10K GeneChip SNP array in the affected siblings, followed by microsatellite analysis, with a multipoint LOD of 2.87. The linkage interval contained one conspicuous candidate gene, *LTBP2*, encoding a latent transforming growth factor-beta binding protein. LTBPs are extracellular matrix proteins with multiple domain structures bearing strong homologies with fibrillins, and may play several roles, including finely controlling TGF- activity in the matrix, a structural role in microfibrils, and a role in cell adhesion. We found a truncating mutation, homozygous in the affected siblings, heterozygous in the parents, and absent from 100 unrelated control subjects from the same ethnic group. Using a polyclonal antibody, we found LTBP2 to be strongly expressed in the calf ciliary zonule. RT-PCR analysis of the proband's skin fibroblasts in primary culture showed absence of the LTBP2 cDNA with no evidence of exon skipping, consistent with nonsense-mediated mRNA decay, contrasting with normal LTBP2 transcript expression in control fibroblasts. We conclude that the *LTBP2* truncating mutation reported here is a rare cause of microspherophakia with marfanoid features. Of interest, LTBP2 has been shown not to bind TGF, and our patients did not show evidence of intense elastolysis or impaired tissue repair as they showed no heart, aortic, skin or lung involvement as encountered in Marfan syndrome.

PRENATAL FINDING OF PERICENTRIC INVERSIONS INVOLVING BOTH CHROMOSOMES 2 AND 9 IN THE SAME PATIENT: TWO CASES WITH NORMAL OUTCOME. *A. Singer[1], J. Rosensaft[2], C. Vinkler[3]* 1) Dept Clinical Gen Unit, Barzilai Med Ctr, Tel Aviv, Israel; 2) Genetic Institute, Kaplan Medical center Rehovot, Israel,; 3) Institute of Medical Genetics, Wolfson Medical Center Holon, Israel.

Pericentric inversion of chromosome 9 [inv(9)(p13;q13)] and pericentric inversion of chromosome 2 [inv(2)(p11;q13)], are both considered polymorphic variations and are the most common forms of autosomal inversion diagnosed prenatally in amniocytes. A small number of cases carrying pericentric inversion of chromosome 9 [inv(9)(p13;q13), were described in association with abnormal ultrasonic findings during pregnancy and rare cases were described with adverse outcome. The homozygote state, of each structural variation, on the other hand, is rarely encountered. In some reports a normal outcome was described while in a few others, an abnormal outcome has been reported. The combination of an inversion of both chromosome 9 and chromosome 2 in the same patient, is uncommon. We present two cases who were diagnosed in utero as carriers of both, the common pericentric inversion on chromosome 9 [inv(9)(p13;q13)] as well as the common pericentric inversion on chromosome 2 [inv(2)(p11;q13)]. In both cases normal babies were born and good outcome reported. One previous case, carrying both inversioms, has been reported and was associated with some fertility problems. Fertility problems have been previously attributed to the common pericentric inversion on chromosome 9 and should be discussed during counseling. Our cases demonstrate that carriers of two inversions are at no increased risk when compared to carriers of each inversion alone and are of generally good outcome.

Genes on chromosomes 1p, 2q, 5q and 6q modify nutritional status in CF. *S. M. Blackman[1,2], L. L. Vanscoy[3], J. M. Collaco[4], K. M. Naughton[2], G. R. Cutting[2]* 1) Pediatric Endocrinology, Johns Hopkins University, Baltimore, MD; 2) Institute of Genetic Medicine, JHU, Baltimore, MD; 3) Pediatrics, National Naval Medical Center, Bethesda, MD; 4) Pediatric Respiratory Sciences, JHU, Baltimore, MD.

Cystic fibrosis (CF) is a Mendelian disease caused by mutations in a chloride channel, CFTR, whose complications include lung disease and pancreatic insufficiency/malabsorption. Nutritional status, a key factor in prognosis, varies widely even among those with the same CFTR mutations, indicating a role for genetic or non-genetic modifiers. To estimate the contribution of genes, height/weight data for 1339 twins and siblings with CF (77,446 visits) were collected by the JHU Twin and Sibling Study. We derived traits reflecting nutrition overall (lifetime mean body mass index (BMI) Z-score; AvgBMI-Z) and before the onset of many CF complications (mean BMI-Z for ages 5 to 7 years; BMI-Z-5to7), and its rate of change (BMI-Z-rate). Concordance for AvgBMI-Z was high in 69 MZ twin pairs (r=0.81) and lower in 15 same-sex DZ twin pairs (r=0.51) and in 145 same-sex sibling pairs within 3 years of age used as a proxy for DZ twins (r=0.49), while trait variance was similar. These twins and siblings estimate heritability at 0.6, indicating that genetic modifiers play an important role in BMI variation among CF patients. Heritability was increased after adjusting for age, cohort, and CF complications (0.7) and was similar for other measures of BMI and for height and weight in CF (0.4-0.7). A subset of this sample (706 CF children, 461 parents) was genotyped with 402 STR markers. Genome-wide linkage analysis revealed evidence for linkage at chromosome 5q15 (LOD=3.78 for BMI-Z-5to7; LOD=1.8 for AvgBMI-Z), one of two loci previously linked to a trait reflecting both pulmonary and nutritional status (Vanscoy et al. 2007. Ped Pulm 42(S30):265 and unpublished data). Linkage was also detected to loci on chromosome 1p36.22 (LOD=4.2, BMI-Z-5to7), 2q33.3 (LOD=3.2, BMI-Z-rate), and 6q23.3 (LOD=3.78, AvgBMI-Z). These results demonstrate that genes other than CFTR play an important role in variation in nutritional status in CF. *Supported by NIH and CF Foundation*.

Hypothalamic-pituitary defects in *Chd7* deficient mice suggest critical roles for *CHD7* in endocrine tissues in human CHARGE syndrome. *D. M. Martin, E. A. Hurd, W. S. Layman* Pediatrics & Human Genetics, University of Michigan Medical Center, Ann Arbor, MI.

CHARGE syndrome is a multiple congenital anomaly condition characterized by ocular coloboma, heart defects, atresia of the choanae, retarded growth and development, genital hypoplasia, and characteristic ear abnormalities. CHARGE has variable phenotypic features which are incompletely penetrant. Many patients with CHARGE display delayed growth, which may be related to early feeding issues, cardiac disease, and/or endocrine dysfunction. Growth hormone (GH) deficiency and other endocrine defects including hypogonadotropic hypogonadism and hypothyroidism have been reported in children with CHARGE. Reduced testosterone levels and pubertal delay are common in boys with CHARGE, and girls display no luteinizing hormone (LH) or follicle-stimulating hormone (FSH) response to GnRH stimulation. CHD7, a member of the CHD family of chromatin remodeling proteins, is mutated in 60-80% of individuals with CHARGE. Members of the CHD family have pivotal roles in chromatin assembly and regulation of gene expression. Based on these observations, we hypothesized that loss of CHD7 disrupts hypothalamic-pituitary signaling during development. To analyze the roles of CHD7 in endocrine function, we generated mice carrying a $Chd7^{Gt}$ allele derived from *Chd7* deficient gene trapped *lacZ* reporter embryonic stem cells. $Chd7^{Gt/+}$ mice exhibit growth delays with onset around postnatal day 7. -galactosidase expression and immunohistochemistry in $Chd7^{Gt/+}$ mice showed *Chd7* expression in the embryonic pituitary and hypothalamus. We found no apparent defects in embryonic pituitary morphology of $Chd7^{Gt/+}$ mice. However, immunofluorescence showed increased GH-positive cells, decreased LH-positive cells, and variable expression of adrenocorticotropin (ACTH) and thyroid-stimulating hormone (TSH) in the late gestation (e18.5) $Chd7^{Gt/+}$ pituitary. Serum IGF-1 was significantly reduced in six week old male $Chd7^{Gt/+}$ mice. These studies suggest that primary defects in hypothalamic-pituitary signaling may underlie the endocrine dysfunction in human CHARGE syndrome.

Bayesian mixture models for case-control genome-wide association studies. *L. Li, A. G. Clark, C. D. Bustamante*
Cornell University, Ithaca, NY.

A fundamental challenge in genome-wide association (GWA) studies is to develop statistical methodology that maximizes power to identify genes responsible for human disease susceptibility while minimizing the number of false positives. Single marker analysis (SMA), involving independent testing of each SNP in a panel for association with a phenotype, has gained wide acceptance in GWA studies due to its simplicity. However, SMA is often limited for complex diseases, since SMA can be significantly biased in estimating effect sizes and significance when the disease is polygenic. We propose a new Bayesian approach to identify multiple quantitative trait loci (QTL) for human complex diseases in case-control GWA studies. The novel approach, based on the logistic or probit model with dimension reduction, simultaneously considers the influence of many SNPs on the disease. SNP effects are assumed to follow 3-component mixture priors, with different truncated normal distributions for positive and negative effects and a large point mass at 0. The point mass at 0 is derived from the assumption that only a small number of SNPs are associated with the disease. With such priors, only significantly nonzero effects have a high posterior probability of remaining nonzero, and modest or negligible effects shrink to 0 with high posteriors. To estimate posteriors, special Gibbs samplers have been developed: an adaptive independence sampler is proposed for the logistic model, and a liability-threshold method is used for the probit model. A score averaging method is proposed to accelerate dimension reduction, making large-scale studies practical. A desktop workstation took 4-6 hours to complete the analysis of human chromosome 1, and 2-4 days for a genome-wide study, while those without the score-averaging acceleration may take months. To compare our approach to SMA, case-control data, based on polygenic models, were simulated from 1,115 Swiss genotyped on the Affymetrix 500k SNP chip as part of the GSK-POPRES project, and Bayesian mixture models, both logistic and probit, as well as SMA were applied. Precision-recall curves suggest our approach is nearly always more powerful than SMA in identifying multiple QTLs, even with the presence of random errors.

Maternal Insulin Secretion or Sensitivity Genes are Associated with Offspring Size at Birth. *M. G. Hayes[1], M. Urbanek[1], L. P. Lowe[1], E. Hughes[1], C. Ackerman[1], N. J. Cox[2], D. B. Dunger[3], A. R. Dyer[1], A. T. Hattersley[4], B. E. Metzger[1], W. L. Lowe[1], HAPO Coop Research Group* 1) Northwestern Univ, Chicago, IL; 2) Univ Chicago, IL; 3) Univ Cambridge, UK; 4) Univ Exeter, UK.

The Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study demonstrated a continuous relationship between maternal glucose measures and birth size or fetal adiposity. We hypothesize genetic factors also contribute to these phenotypes, and examined genetic variation in loci previously implicated in insulin secretion or sensitivity to determine associations with maternal glycemia and insulin secretion at ~28 weeks gestation and/or offspring size at birth for 2133 Thai HAPO mothers and their offspring. We investigated the association of fetal genotype with birth outcomes [birth weight (BW), birth length (BL), head circumference (HC), and sum of skinfolds (SSF)], and maternal genotype with these birth outcomes as well as maternal traits [fasting glucose (FG) and C-peptide (FCP) and 1-hr glucose (1hG) from the oral glucose tolerance test]. Associations were assessed through linear regressions with the single trait/outcome under an additive genetic model adjusting for known confounders. We found significant associations ($p<0.01$) between maternal FG or 1hG and maternal SNPs: *ABCC8* (best SNP is rs2073583; p=0.004 1hG), *GCK* (rs917793; p=0.002 FG), *HNF4A* (rs2071200; p=0.004 1hG), *PPARG* (rs4498025; p=0.009 FG), and *TCF7L2* (rs290484; p=0.009 FG). In a subset of these genes, fetal SNPs were associated with fetal BL (*PPARG* rs2972164, p=0.004), or SSF (*ABCC8* rs1055574, p=0.007). Further, several SNPs were associated with multiple maternal and/or fetal traits. Each maternal copy of the major G allele for *PPARG* rs4498025 was associated with increased FG (0.8mg; p=0.009) and FCP (0.06g; p=0.04), while each fetal copy of this allele was associated with decreased BW (43g; p=0.01). Each maternal copy of the minor G allele for *ABCC8* rs2237975 was associated with increased FG (0.5mg; p=0.04), SSF (0.2cm; p=0.009), HC (0.09cm; p=0.02), BL (0.1cm; p=0.009), and BW (42g; p=0.001). These results indicate genes involved in insulin secretion or sensitivity also impact birth size or fetal adiposity.

Worldwide genetic structure in 37 genes important for telomere maintenance using HGDP and HapMap data. *L. Mirabello[1], N. Xiao[2], X. Deng[2], L. Qi[2], Z. Wang[2], S. A. Savage[1]* 1) Clinical Genetics Branch, DCEG, NCI, National Institutes of Health; 2) SAIC-Frederick, and Core Genotyping Facility, DCEG, NCI, National Institutes of Health.

Telomeres form the ends of eukaryotic chromosomes and are vital in maintaining genetic integrity and function by keeping the chromosomes intact upon replication, preventing end-to-end fusion and atypical recombination. Telomere dysfunction has been linked to many diseases, including bladder and lung cancers, diabetes mellitus, cardiovascular disease, Alzheimers disease, and ulcerative colitis. We investigated patterns of differentiation and the haplotype structure of 37 genes important for telomere maintenance among 53 populations from sub-Saharan Africa, North Africa, the Middle East, Europe, Central/South Asia, East Asia, Oceania, and the Americas. Data from 1168 unrelated individuals was obtained from the genome-wide scan (650,000 common SNPs) of the Human Genome Diversity Panel (HGDP-CEPH) and from the HapMap database (Phase II) at 716 single-nucleotide polymorphism loci. Estimated haplotype frequencies, measures of genetic diversity, linkage disequilibrium (LD), population differentiation and structure were computed. The greatest gene diversity was observed in Africa, as anticipated. The majority of genes had low to moderate haplotype diversity; the lowest was observed in *DKC1*, *TERC*, *XRCC6* and the greatest in *RAD51L1*, *TEP1*, and *TERT*. Overall, there was significant differentiation between continental regions; the haplotype composition and LD patterns also varied among the regions. Structure analyses partitioned worldwide diversity into five genetic clusters that correspond to Sub-Saharan Africa, Eurasia, East Asia, America, and Oceania. The population of Utah residents from the USA partitioned into the Eurasia cluster. Although some telomere genes have higher than expected haplotype diversity (*e.g. TERT*), as a group there appears to be less diversity overall in these genes than in other sets of genes with different functions. Higher levels of genetic variation may not be tolerated in these genes, possibly due to their critical role in telomere maintenance and chromosomal stability.

Genetic factors contribute to dental caries in the primary and mixed dentitions in Appalachian children. *X. Wang[1], S. Wendell[1], G. Barkanic[1], R. J. Weyant[1], R. Crout[2], D. McNeil[2], M. L. Marazita[1]* 1) School of Dental Med, Univ Pitt, Pittsburgh, PA; 2) School of Dentistry, West Virginia Univ, WV.

The prevalence of dental caries (tooth decay) in children has dramatically increased in the past years, resulting in a major public health concern in children, and heavy economic burdens to society. Dental caries is a multifactorial complex disease, due to interactions between genetic, environmental and behavioral factors. We hypothesize that genetic factors play a crucial role in controlling both susceptibility and resistance to this disease. Two dental caries phenotypes based on the location of the decay (dmfs-pit/fissure, and dmfs-smooth surface) were assessed in 1382 participants (age15) from 618 families from the Center for Oral Health Research in Appalachia (COHRA). The Family Based Association Test (FBAT) was used to test associations with 29 SNPs from 24 caries candidate genes, in two non-overlapping subsets: primary dentition (age6) and mixed dentition (6age15). Further, we evaluated each caries phenotype twice for assessing caries susceptibility alleles (transmission patterns to children with caries) and caries resistance allele (transmission to children with no caries) separately. For caries scores of both pit/fissure and smooth surfaces (in both primary and mixed dentitions), 5 SNPs in 3 taste receptor genes (TAS1R1, TAS1R2 and TAS2R38), were consistently associated with protection against tooth decay (0.007P0.1). Similarly, one SNP from the cathepsin B gene (CTSB) demonstrated consistent association with risk for caries for both dentitions and phenotypes (0.05P0.1). In addition, SNPs from 2 genes (AMELX and CALR) were associated only with risk of pit/fissure caries (P0.05), but not with smooth surface caries. Notably, one allele (G allele) in CALR SNP was significantly associated with caries risk, while the opposite allele (T allele) showed borderline association with protection (p=0.07). Our results indicate that genetic factors are associated with caries risk and protection in children. Further, tooth decay on pit/fissure and smooth surfaces are associated with both common and unique sets of genes. NIH Grant # DE014899.

Defining UBE-1 Mutations and Ubiquitination Defects Underlying X-linked SMA (XL-SMA). *L. Baumbach[1], J. Ramser[2], M. E. Ahearn[1], K. O. Yariz[1], C. Lenski[2], B. Burnett[3], L. Wan[4], G. Dreyfuss[4], K. Fischbeck[3], A. Meindl[2]* 1) Univ Miami Sch Medicine, Miami, FL; 2) Technische Universitaet Muenchen, Munich, Germany; 3) NIH, Bethesda, MD; 4) Univ Penn Sch Medicine, Philadelphia, PA.

Our group has described an X-linked form of lethal infantile SMA (MIM 301830) with additional features of early onset/congenital contractures/fractures. We have identified the first human UBE-1 disease alleles in five unrelated X-linked SMA families. UBE-1 (Ubiquitin-Activating Enzyme E1) catalyzes the first step in the ubiquitin conjugation cascade. Two of the XL-SMA mutations are missense substitutions in highly conserved amino acids of UBE-1; the third mutation is a recurrent SNP which was shown to alter UBE1 expression (Meindl et al., this meeting). Co-segregation of these mutations with disease has been confirmed in the families, and not detected in control X chromosomes.. We have been investigating downstream effects on the ubiquitination-proteasome (UPP) pathway and SMN complex stability. SMN and other members of the SMN complex are quantitatively being measured in cell lines from XL-SMA, SMA Type I patients and controls using Western blots quantitative immunoassays, and a SMN complex activity assay. RNA expression levels of SMN and selected Gemins are being analyzed by Taqman assays. A series of related experiments are being completed which will predict in vivo effects of human UBE-1 mutations, including in vitro measurements of UBE-1 activity and downstream ubiquitination defects, evaluation of proteasome activity, and immunohistochemical analysis of UBE-1 protein. Western blot analyses and immunoassays suggest that UBE-1 protein is 20-30% reduced in XL-SMA patients, while SMN protein is 60% reduced due to an unidentified mechanism. SMN turnover and SMN complex assembly appear not to be affected in XL-SMA patients, and no direct effects of UBE-1 mutations have been demonstrated on ubiquitin activation or gross UPP activity.. Our evidence suggests that XL-SMA is part of a growing list of neurodegenerative disorders associated with defects in ubiquitination, and that XL-SMA and SMA share a common biological pathway which results in reduction of the SMN complex.

Renal phenotypes related to TCF2/HNFIB rearrangements diagnosed antenatally. *K. Dahan[1], N. Godefroid[2], L. Collard[3], A. Destree[4], M. Lizon[1], W. Courtens[1], O. Devuyst[2], Y. Pirson[2]* 1) Ctr Human Genetics, Univ Catholique Louvain, Brussels, Belgium; 2) Division of Nephrology, Université Catholique de Louvain, Brussels, Belgium; 3) CHC St Vincent Pediatric Nephrology, Rocourt, Belgium; 4) Centre de Génétique Humaine, Institut de Pathologie et de Génétique, Gosselies.

Mutations in TCF2 are known to be the cause of Maturity-Onset Diabetes of the Young type 5, a dominantly inherited multisystem developmental disorder with diabetes, pancreas atrophy, liver involvement, urogenital malformations. Whereas classical MODY5 is easily diagnosed, those apparently restricted to renal phenotype may be under recognized, especially in antenatal period. Although TCF2 genomic deletions are the most frequent cause of bilateral hyperechogenic kidneys, its impact on renal function remains unclear. Here, we summarize features of 9 children (6 girls;3 boys) and two adult relatives (2 males) from 8 families. The TCF2 mutation was whole gene deletion in 6/8, exon 6 deletion in 1/8 and exon 5 duplication in 1/8; de novo mutation was present in 6/8 families. All affected children had antenatally diagnosed hyperechogenic bilateral kidneys. After birth-median age at last follow was 28 mo (range 0 to 96mo)-, we found bilateral multicystic dysplasia in 1/9, renal cysts in 9/9 (bilaterally in 9/9), and renal dysplasia in 5/9 (bilaterally in 3/5). Three children had renal failure leading to ESRD at median age of 40 mo (range 0 to 92 mo). Of them, two were of low birth weight and one had prenatal renal failure with severe oligohydramnios diagnosed at 34wk of gestation. Pancreas size was normal by sonographic evaluation in the 8 tested and one had developed diabetes after renal transplantation. Extrarenal features compromised jejunum stenosis in 1/9, elevated liver enzymes in 3/4, hyperuricemia in 3/6 and macrocephalia in 1/8. Among the 2 adults (mean age of 31 y), we found bilateral medullary cysts in 1/2, dysplastic kidneys in 1/2, and progressive renal failure in 2/2 leading to ESRD in 1/2 at age 30. None had diabetes. These data illustrate the large clinical variability among TCF2 patients even in presence of identical molecular defect and common antenatal presentation.

Defects in neural stem cell proliferation in *Chd7* deficient mice suggest that olfactory epithelial dysfunction contributes to anosmia in human CHARGE syndrome. *W. S. Layman[1], D. P. McEwen[3], L. A. Beyer[4], S. R. Lalani[5], S. D. Fernbach[5], J. W. Belmont[5], Y. Raphael[4], J. R. Martens[3], D. M. Martin[1,2]* 1) Human Genetics, Univ of Michigan; 2) Pediatrics, Univ of Michigan; 3) Pharmacology, Univ of Michigan; 4) Otolaryngology, Univ of Michigan; 5) Genetics, Baylor College of Medicine.

Haploinsufficiency for CHD7, a chromodomain protein, causes CHARGE syndrome, a multiple anomaly disorder characterized by ocular coloboma, heart defects, atresia of the choanae, retarded growth and development, genital hypoplasia, and ear anomalies. The clinical features of CHARGE syndrome are highly variable and incompletely penetrant, but reduced olfaction and defects in the olfactory bulb have been reported. CHD7 is mutated in 60-80% of individuals with CHARGE. Members of the CHD family have pivotal roles in chromatin assembly and regulation of gene expression. Based on these observations, we hypothesized that loss of *Chd7* disrupts mammalian olfactory tissue development. We found severe defects in olfaction in children with *CHD7* mutations and CHARGE using the Basic Smell Identification Test (B-SIT). To analyze the role of CHD7 in olfaction, we generated mice carrying a $Chd7^{Gt}$ allele derived from *Chd7* deficient gene trapped *lacZ* reporter embryonic stem cells. Olfactory function analyzed by electro-olfactogram recordings in adult littermate mice showed that $Chd7^{Gt/+}$ mice have severely impaired olfaction independent of the olfactory bulb. -galactosidase expression in the $Chd7^{Gt/+}$ embryonic olfactory epithelium and immunofluorescence with an anti-CHD7 antibody in adult olfactory epithelium showed that *Chd7* is expressed in both sustentacular cells and basal (olfactory progenitor) cells but not olfactory sensory neurons. BrdU incorporation assays showed a significant (50%) reduction in proliferating CHD7-positive cells in the mature $Chd7^{Gt/+}$ olfactory epithelium. These studies suggest that olfactory dysfunction caused by reduced *Chd7* function in the mouse may be attributed to intrinsic defects in olfactory neural stem cell proliferation, which contribute to the olfactory defects associated with human CHARGE syndrome.

Variants in the ACAD10 Gene are Associated with Type 2 Diabetes, Insulin Resistance and Lipid Oxidation in Pima Indians. *L. Bian, Y. Muller, L. Ma, R. Hanson, S. Kobes, C. Bogardus, L. Baier* PECRB, NIDDK, NIH, Phoenix, AZ 85004.

A prior genome wide association (GWA) study in Pima Indians identified a single nucleotide polymorphism (SNP) within the ACAD10 gene that was associated with early-onset type 2 diabetes (T2D). ACAD10 encodes an acyl-CoA dehydrogenase that catalyzes mitochondrial fatty acid beta-oxidation, a key event responsible for insulin resistance and T2D. Therefore, ACAD10 was analyzed as a prior candidate gene for T2D. A total of 21 SNPs, identified by sequencing or selected from database, were genotyped in 895 case/control subjects for early-onset T2D as well as 415 non-diabetic individuals for predictors of T2D. All variants were further genotyped in 3501 full-heritage Pima Indians. Linkage-disequilibrium (LD) analysis showed that all of the 21 SNPs fell into one LD block and 3 representative SNPs (rs7136874, rs659964, rs601663) could capture all of the genetic information. SNPs represented by rs7136874 were associated with T2D (adjusted P = 0.003-0.006) among the case/control subjects. The diabetic risk alleles were associated with a decreased rate of insulin-stimulated glucose disposal at both physiological and maximally stimulating insulin concentrations during the clamp(adjusted P = 0.05-0.15 and 0.005-0.01, respectively), and a reduced rate of basal lipid oxidation (adjusted P = 0.02-0.03), among the non-diabetic individuals. SNPs represented by rs659964 showed the strongest associations with T2D (adjusted P = 0.000002-0.00001). Similarly, the diabetic risk alleles were significantly associated with reduced insulin sensitivity during the high dose insulin clamp (adjusted P = 0.002-0.01) and decreased basal lipid oxidation (adjusted P = 0.02-0.07) among the non-diabetic subjects. SNPs in perfect LD with rs601663 showed a marginal association with both T2D (adjusted P = 0.01-0.05) and reduced insulin sensitivity during the clamp (adjusted P = 0.01-0.06). In the population-based analysis of 3501 full-heritage Pima Indians, SNPs in perfect LD with rs659964 remained significantly associated with T2D (adjusted P = 0.0006-0.001). Our data indicate variants in ACAD10 may increase susceptibility to T2D by impairing insulin sensitivity via abnormal lipid oxidation.

CRISPLD1: Expanding the role of CRISPLD genes in Nonsyndromic Cleft Lip with or without Cleft Palate. *B. T. Chiquet[1,2], R. Henry[1], D. Ma[3], A. Burt[3], J. B. Mulliken[4], S. Stal[5], S. H. Blanton[3], J. T. Hecht[1]* 1) Dept Pediatrics, Univ Texas Med Sch, Houston, Houston, TX; 2) Univ Texas Dental Branch, Houston, TX; 3) Univ Miami Miller School of Medicine, Miami, FL; 4) Children's Hospital, Boston, MA; 5) Texas Children's Hospital, Houston, TX.

Nonsyndromic cleft lip with or without cleft palate (NSCLP) is a common complex birth defect caused by genes and environmental factors. Despite having substantial genetic liability, less than 15 percent of the genetic contribution to NSCLP has been identified. We recently found that variation in CRISPLD2 (cysteine rich secretory protein LCCL domain 2) is associated with NSCLP and that CRISPLD2 is expressed in developing murine craniofacies. Our recent genome scan found suggestive linkage of NSCLP (LOD1.0) to 8q13.2-21.13, which contains the CRISPLD1 gene. Though little information is known about either CRISPLD gene, both protein products contain more cysteines than comparably sized proteins. The folate pathway is responsible for endogenous cysteine production; variation in four folate pathway genes have previously been associated with NSCLP. We hypothesized that variation in the CRISPLD1 gene contributes to NSCLP and that both CRISPLD genes interact with genetic variants in the folic acid pathway. CRISPLD1 SNPs were genotyped in nonHispanic white and Hispanic NSCLP families. Association was detected between NSCLP and CRISPLD1 in the total nonHispanic white sample (6 SNPs, 0.01p0.05) and in those without a family history of NSCLP (5 SNPs, 0.005p0.05). Altered transmission of CRISPLD1 haplotypes were found in both ethnicities (0.003p0.05). Evidence for a gene-gene interaction was not detected between the two CRISPLD genes; however, gene-gene interaction was detected between CRISPLD genes and folic acid pathway genes (0.002p0.05), including 5,10-methenyltetrahydrofolate synthetase, which was significant in both ethnicities. This data suggests that variation in both CRISPLD genes play a role in NSCLP, but not through a detectable interaction. Variation in each CRISPLD gene interacts with genetic variants in folic acid pathway genes. This is an important observation and may explain a novel etiologic NSCLP pathway.

Detailed Characterization of the Dyskeratosis Congenita Phenotypic Spectrum. *C. M. Mueller[1], N. Giri[1], G. Seratti[2], B. P. Alter[1], S. A. Savage[1]* 1) CGB/DCEG/NCI/NIH, Bethesda, MD; 2) MGB/NHGRI/NIH, Bethesda, MD.

Dyskeratosis congenital (DC) is characterized by the diagnostic triad of dysplastic nails, lacey skin pigmentation, and oral leukoplakia, but diverse clinical features have been reported. Individuals with DC are at high risk of developing aplastic anemia, myelodysplastic syndrome (MDS), leukemia and epithelial cancers. 5 telomere biology genes are known to cause DC; some individuals have no identifiable mutation. The unifying feature is extremely short telomere length, 1st%-ile; for age, in leukocyte subsets. Our objective was to comprehensively characterize the DC phenotype. We systematically evaluated 32 individuals(22 families) with either a known mutation in a DC gene, the classic DC phenotype or very short telomeres and a family member with classic DC from NCIs Inherited Bone Marrow Failure Syndromes protocol: including history, physical and dysmorphology examinations, as well as neuro-otology, dermatology, dental, otolaryngology and ophthalmology evaluations. We studied 25 males and 7 females, median diagnosis age 9 years (0-28). Mutation status: 5 DKC1(16%), 3 TERC(9%), 1 TERT(3%), 11 TINF2(34%), and 12 unknown(38%). 18(56%) had 2 features of the diagnostic triad; 20(63%) dysplastic nails, 16(50%) skin pigmentation and 19(59%)oral leukoplakia. 29(91%) had bone marrow failure, 1(3%)MDS and 2(6%)epithelial cancers. We found abnormalities in several systems more frequently than previously reported, including cerebellar hypoplasia(31%), ataxia(22%), developmental delay(50%); dental anomalies(56%); and pulmonary function abnormalities(56%). No distinct facies or skeletal dysplasias were observed. One subject had only pulmonary findings and another no clinical features. Preliminary analyses do not suggest genotype/phenotype correlations. The phenotypic spectrum of DC in our cohort ranges from severely affected to minimally affected. We observed a higher frequency of neurologic, dental and pulmonary abnormalities in our series. The DC phenotype is broader than is widely recognized. Comprehensive evaluation of all individuals, regardless of whether they meet the classic diagnostic triad, is necessary to better understand the clinical consequences of DC.

Mutation screening of CHD5 in melanoma-prone families linked to 1p36 revealed no pathologic changes. *D. Ng, X. Yang, M. Tucker, A. Goldstein* Genetic Epidemiology Branch, DCEG/NCI/NIH, Rockville, MD.

Background: A subset of cutaneous malignant melanoma and dysplastic nevi (CMM/DN) families is linked to 1p36. To date, no CMM/DN susceptibility gene has been identified at this locus. Data from mouse studies identified chromodomain helicase DNA binding protein 5 (CHD5) as a tumor suppressor gene affecting cellular proliferation and apoptosis via the CDKN2A/p53 pathway. Based on these findings, we felt it was important to screen CHD5 as a familial CMM/DN susceptibility gene. Methods: Eight unrelated CMM/DN families showing prior evidence of linkage to the 1p36 locus were identified for CHD5 mutation screening. One CMM/DN affected and one unaffected individual from each family were selected for sequencing of the coding exons and their respective intron-exon boundaries contained in CHD5. CHD5 variants that were identified solely among affecteds in the screening panel were further assessed by sequencing additional affected and unaffected members of these families to determine if the variant co-segregated with the CMM/DN phenotype. Results: Single nucleotide polymorphisms in the CHD5 intronic and coding regions were identified among affecteds in the screening panel. None of these variants completely co-segregated with CMM/DN affection status among these eight families. Conclusion: There is no evidence to support CHD5 as a major melanoma susceptibility gene among the eight CMM/DN families screened.

Feasibility and relevance of examining lymphoblastoid cell lines to study role of microRNAs in autism. *Z. Talebizadeh, M. Theodoro* Childrens Mercy Hospital and University of Missouri-Kansas City, Kansas City, MO.

Noncoding RNAs (ncRNAs) such as microRNAs do not code for protein but may regulate gene expression. microRNAs are small RNA molecules that regulate the expression of genes by binding to the 3-untranslated regions of specific mRNA directing translational repression or transcript degradation. Multiple classes of ncRNAs are highly represented in the nervous system, emphasizing the likelihood that nervous system development and function is heavily dependent on RNA regulatory network. Despite growing evidence for regulatory influence of ncRNAs in gene expression, particularly in brain function, this group of regulatory factors has not been evaluated in autism spectrum disorders (ASD), the most common childhood neurodevelopmental disease. To assess the feasibility and relevance of utilizing lymphoblastoid cell lines (LCL) to examine the role of microRNAs in autism, we performed a pilot study. Subjects were ascertained from the Autism Genetics Resource Exchange. Global expression profiling of 470 mature human microRNAs were examined using LCL derived RNA (LC Sciences, Houston, TX) from 6 subjects with autism compared with 6 age-gender matched unaffected subjects. Differential expression (either higher or lower) for 9 of the 470 microRNAs was observed in our autism samples compared with controls. Potential target genes for these microRNAs were identified using publicly available programs. Some microRNAs are tissue-specific while others are more abundant. Our pilot study represents an important first step in the possibility of utilizing LCL to identify at least a subset of microRNAs differentially expressed in autism subjects with a potential functional relevance. In the absence of having access to a large autism brain samples, evaluating LCL samples would still be informative in detecting changes for a subset of brain-expressed microRNAs implicated in autism. Subsequently, this model system should allow for detection of complex subtle changes in susceptibility genes/pathways contributing to autism. Genome-wide expression profiling is underway to examine the correlation between differentially expressed microRNAs and their potential target mRNAs.

Germline genetic variants associated with breast cancer survival in a genome-wide association study of breast cancer susceptibility. *E. Azzato[1,2], J. Tyrer[2], D. Greenberg[3], D. Easton[4], N. Caporaso[1], P. Pharoah[2], Breast Cancer Association Consortium* 1) Division of Cancer Epidemiology and Genetics, NCI, Rockville, MD; 2) Oncology Dept, Cambridge Univ, Cambridge, UK; 3) Eastern Cancer Registration and Information Centre, Cambridge, UK; 4) Cancer Research UK Genetic Epidemiology Unit, Cambridge, UK.

Somatic alterations correlate with breast cancer prognosis and survival, but less is known about the effects of common inherited genetic variation. We evaluated the association between survival after breast cancer diagnosis and 12,711 single nucleotide polymorphisms (SNPs) genotyped as part of a 2-stage genome-wide association study of breast cancer susceptibility. We obtained genotype data for 3,820 women with invasive breast cancer in SEARCH, a population-based case-control study. We tested polymorphisms for association with all-cause mortality (n=542) using Cox regression analysis and a one degree of freedom trend test. For replication, we obtained genotype and follow-up data for 11,161 women diagnosed with invasive breast cancer (1,432 deaths) from 13 studies in the international Breast Cancer Association Consortium and performed a fixed-effects meta-analysis. We genotyped two SNPs in the replication based on strong associations with survival in SEARCH ($p10^{-5}$). One SNP (rs4778137), located in the oculocutaneous albinism II gene (OCA2), showed a weak non-significant protective effect in the replication (replication HR=0.95, 95% CI: 0.87-1.04, p=0.26; pooled HR=0.89, 95% CI: 0.82-0.96, p=0.001). We found evidence that rs4778137 varies by ER status (p_{het}=0.01) and that the protective effect of this SNP appears limited to ER negative tumors (replication HR=0.83, 95% CI: 0.70-1.00, p=0.046; pooled HR=0.76, 95% CI: 0.65-0.89, p=0.001). The other SNP (rs6626269), located approximately 300 kilobase pairs upstream from the fragile X mental retardation 1 gene (FMR1), did not replicate (replication HR=0.99, 95% CI: 0.89-1.10, p=0.75; pooled HR=1.13, 95% CI: 1.04-1.23, p=0.004). There is some evidence that rs4778137 (OCA2) or a variant in linkage disequilibrium may be associated with prognosis after a diagnosis of ER negative breast cancer.

Molecular investigation of TBX1 gene in Midline Facial Defects with Hypertelorism. *M. Simioni, E. L. Freitas, I. Lopes-Cendes, V. L. Gil-da-Silva-Lopes* Medical Genetics, FCM/UNICAMP, Campinas, Brazil.

Midline facial defects with hypertelorism (MFDH) are a group of rare and heterogeneous condition involving anomalies of frontonasal process. Structural and functional anomalies of the central nervous system are also present. These CNS abnormalities have similarity with those found in patients with 22q11.2 deletion syndromes. Mutations in TBX1 gene (22q11.2 region) are also described in individuals with these conditions. There are some isolated reports of MFDH in which 22q11.2 deletion were detected. These facts suggested that some cases of MFDH may be part of the spectrum of these conditions and associated to sequence alterations in TBX1 gene. In order to verify this hypothesis, 10 individuals with MFDH were screened for mutations in this gene. All of them were previously investigated by clinical dysmorphologic and magnetic resonance of central nervous system. We selected individuals with normal karyotype (46,XX or 46,XY) and individuals that do not presented 22q11.2 deletion, previously investigated by FISH technique. Several alterations, classified as single nucleotide polymorphism (SNP) at databases such as NCBI, were found. A nucleotide substitution G A at position 297 (A99A), previously reported as a common polymorphism and as a rare variant, was found in one individual. A new alteration 1132G A that causes an amino acid substitution at codon G378S was found in one individual. Although the sample could be considered relevant as the rarity of these defects, in view of the size of the sample, the clinical-genetic heterogeneity and the new alteration found, we could not exclude neither associate the involvement of 22q11.2 region in the pathogenesis of MFDH. For these reasons, we consider that each situation of MFDH should be considerate individually in order to provide an adequate clinical follow-up for patients. Financial support: FAPESP.

Seeing the trees to understand the forest: array comparative genomic hybridization of 49 subjects with a Smith-Magenis-like phenotype. *S. Williams[1], S. Girirajan[1], D. Tegay[3], N. Nowak[4], E. Hatchwell[5], S. Elsea[1,2]* 1) Dept Human Genetics, Virginia Commonwealth University, Richmond, VA; 2) Dept of Pediatrics, Virginia Commonwealth University, Richmond, VA; 3) Dept of Medicine, New York College Osteopathic Medicine, Old Westbury, NY; 4) Dept of Cancer Prevention and Population Sciences, University of Buffalo, NY; 5) Depts of Genetics and Pathology, SUNY, Stony Brook, NY.

Smith-Magenis syndrome (SMS) is caused by del(17)p11.2, including the retinoic acid induced 1 gene (RAI1) or mutation of RAI1. Haploinsufficiency of RAI1 results in developmental delay, mental retardation, sleep disturbance, self-abusive behaviors, and most features commonly seen in SMS. Our lab has a cohort of ~60 subjects who were referred for molecular analysis due to an SMS-like phenotype. Within this cohort, deletion and mutation analyses of RAI1 were negative; thus, the clinical diagnosis of SMS cannot be confirmed and suggests that at least one other locus is responsible for the phenotype observed. Here we present whole-genome array comparative genomic hybridization of 49 subjects whose phenotypes have significant overlap with that of SMS. Specifically, this SMS-like cohort exhibits developmental delays, sleep disturbance, self-abusive behaviors, motor dysfunction, and hyperactivity of the same type and prevalence as that of SMS. From this study, we have discovered at least 19 new loci that are likely to contribute to the SMS-like phenotype. Genes in these regions contribute to development, neurological fidelity, and morphology, all of which are affected in SMS. In addition, as a result of the phenotypic overlap between SMS and SMS-like patients, these data may provide some insight into the function of RAI1, including the pathways in which it may be involved and the genes it may regulate. These data will improve diagnosis, understanding, and potentially treatment of these complex behavior and mental retardation syndromes.

Pre-B-Cell Leukemia Homeobox 1 (PBX1) Shows Genetic and Functional Association with Bone Mineral Density Variation. *C. L. Cheung[1,6], B. Y. Chan[1], V. Chan[1], S. Ikegawa[4], I. Kou[4], H. Ngai[1], D. K. Smith[2], Q. Y. Huang[1], S. Mori[5], P. C. Sham[3], A. W. Kung[1]* 1) Departments of Medicine; The University of Hong Kong, Pokfulam, Hong Kong; 2) Departments of Biochemistry; The University of Hong Kong, Pokfulam, Hong Kong; 3) Departments of Genome Research Centre; The University of Hong Kong, Pokfulam, Hong Kong; 4) Laboratory for Bone and Joint Disease, Center for Genomic Medicine, RIKEN, Tokyo, Japan; 5) Department of Internal Medicine, Tokyo Metropolitan Geriatric Hospital, Tokyo, Japan; 6) Current address: IFAR, Hebrew SeniorLife, Harvard Medical School, Boston, MA, USA.

Bone mineral density (BMD) is one of the major determinants of risk for osteoporotic fracture. Multiple studies reveal that peak bone mass is under strong genetic influence. One of the major susceptibility loci for peak spine BMD has been mapped to chromosome 1q21-q23 in the Caucasian population. We have previously replicated this finding in Southern Chinese pedigrees and detected a maximum multipoint LOD score of 2.36 at D1S196. To further fine-map this region, 380 single nucleotide polymorphic (SNP) markers were genotyped in 610 sibpairs from 231 families. Several markers were identified in the association analysis as important candidates underlying BMD variation. Among them, successful replication was demonstrated for SNPs in pre-B-cell leukemia homeobox 1 (PBX1) gene in two other unrelated Southern Chinese and Japanese case-control cohorts. The functional role of PBX1 in bone metabolism was examined in vitro using human bone derived cells (HBDC) and murine MC3T3-E1 pre-osteoblasts. PBX1 mRNA was constitutively expressed in both HBDC and MC3T3-E1 cells. Immunostaining revealed that PBX1 is localized in the nucleus compartment. Silencing of PBX1 by RNAi in MC3T3-E1 cells decreased the expression of Runx2 and Osterix, the critical transcription factors for osteogenesis, but accelerated cell proliferation and bone nodule formation. Overall our data support a genetic and functional association of PBX1 with BMD variation.

GKAP and GRIN2B are associated with full scale IQ in AD/HD families. *C. A. Markunas[1], K. Quinn[1], A. L. Collins[1], M. E. Garrett[1], S. Keatts[1], A. M. Lachiewicz[2], E. Morissey-Kane[3], S. H. Kollins[4], A. D. Anastopoulos[3], A. E. Ashley-Koch[1]* 1) Center for Human Genetics, Duke Medical Center, Durham, NC; 2) Department of Pediatrics, Duke Medical Center, Durham, NC; 3) Department of Psychology, University of North Carolina, Greensboro, NC; 4) Department of Psychology, Duke Medical Center, Durham, NC.

The postsynaptic density (PSD) is a cytoskeleton specialization attached to the postsynaptic membrane. It contains a complex protein signaling network, thought to be involved in information processing and memory formation. We evaluated a subset of PSD genes, including GKAP, SHANK3, NMDAR subunits 1-5, and AMPAR subunits 1-4, in a family-based association study. The dataset included 104 families with at least one child (5-12 yrs) meeting research criteria for AD/HD. Full scale IQ (FSIQ) and working memory indices (WMI) were obtained from the WAIS-III (parents) or the WISC-IV (affected and unaffected children) intelligence tests. Genotyping was performed using the Illumina Infinium HumanHap300 duo chip. A minimal number of tagging SNPs in each gene were selected for analysis (N_{total}=252). Using QTDT, each SNP was tested for association with FSIQ and WMI. One intronic SNP (rs11663827) in GKAP was significantly associated with FSIQ (nominal $p<0.0001$) and remained significant after applying an FDR-adjusted p-value threshold of 0.05. Using a less stringent FDR threshold of 0.20, 4 additional intronic GKAP SNPs flanking rs11663827 remained significantly associated with FSIQ, as well as 3 intronic SNPs in GRIN2B (NMDAR subunit). Two significant SNPs (GKAP: rs1791397, GRIN2B: rs1158541) were predicted by PupaSuite to be putative triplex disrupting SNPs and may affect gene expression. WGAViewer was used to evaluate the cis-effect of each significant SNP, and any SNP within the same HapMap LD bin ($r^2>0.7$), on GKAP or GRIN2B expression levels (GENEVAR dataset). In Caucasian children, but not parents, 75% of the SNPs present in the LD bin with rs1008619 were nominally associated ($p<0.05$) with GRIN2B expression levels. Our results support further investigation of GKAP, GRIN2B, and additional PSD genes in relation to learning and memory formation.

The autism phenotype in different racial-ethnic groups. *M. L. Cuccaro[1], J. Lee[1], K. Hamilton[1], R. K. Abramson[2], H. H. Wright[2], J. R. Gilbert[1], M. A. Pericak-Vance[1]* 1) Dept Medicine, Univ Miami Sch Med, Miami, FL; 2) School of Medicine, Univ South Carolina, Columbia, SC.

Autism is a complex disorder which affects individuals of all racial-ethnic groups. Using different populations has been successful in disentangling genetic complexity in complex diseases such as prostate cancer and asthma. We have established collections of African American (AA) and Hispanic American (HA) autism families for genetic analyses. In this study we compared our AA (n=75), HA (n=75), and Caucasian (CA n=171) autism samples on clinical-phenotypic measures including the Autism Diagnostic Interview-R (ADI-R), Aberrant Behavior Checklist (ABC), Vineland Adaptive Behavior Scales (VABS) and Social Responsiveness Scale (SRS). Most striking were group differences in ADI-R derived language traits including *age at first words* (p = 0.0005) and *age at phrase speech* (p 0.0001). Both the AA and HA groups were significantly delayed relative to the CA group. A clear relationship between functional language level and racial-ethnic group was identified as both the AA and HA groups had higher proportions of the individuals with no functional language (^{2}=29.22, df=2, p 0.0001). Group differences in adaptive level, another developmental indicator, were noted as well. No group differences were detected in the ADI-R domain scores, the SRS, or ABC scales suggesting that the language differences were not simply due to severity. Our findings are consistent with our previously published work suggesting a potential developmental language phenotype in AA and HA autism groups. This phenotypic variation may reflect differences in underlying autism risk genes in these racial-ethnic groups. These findings suggest that the autism phenotype in AA and HA families needs to be examined more closely as they may have implications for genetic analyses.

Contribution of a deep intronic mutation to Pelizaeus-Merzbacher disease. *J. Taube[1], L. Banser[1], S. Driscoll[1], G. Mancini[2], M. Meuwissen[2], C. Catsman[2], E. Sistermans[3], J. Garbern[4], G. Hobson[1]* 1) Nemours Biomedical Research, duPont Hosp Child, Wilmington, DE; 2) Erasmus University MC/Sophia Childrens Hospital, Rotterdam, Netherlands; 3) VU Univ Med Ctr Amsterdam, Amsterdam, Netherlands; 4) Wayne State University, Detroit, MI.

Pelizaeus-Merzbacher Disease (PMD) is a neurological disease often caused by duplication of the proteolipid protein 1 gene (*PLP1*) on the X-chromosome. PMD is also caused by complete deletion *PLP1*, as well as small deletions, insertions and single base mutations that affect the coding sequence of the protein or the splicing of the gene. The gene has two major spliced forms due to alternative 5' splice donor sites for intron 3: *PLP1* at c.453 and *DM20* at c.348. *PLP1* expression is largely restricted to oligodendrocytes in the brain, while *DM20* is more widely expressed. Our lab has demonstrated previously that mutations within the *PLP1* intron 3 donor site reduce expression of the *PLP1* splice form both in patient fibroblasts and in transfections of mini-gene constructs into cultured cells. Presently we are investigating the effects on the *PLP1/DM20* splicing ratio of two intronic mutations identified in PMD patients: IVS3+7A>G and IVS3-322G>A. We used site-directed mutagenesis to create these patient mutations in our mini-gene splicing construct and analyzed splicing by semi-quantitative RT-PCR after transfection into an oligodendrocyte-like cell line, Oli-neu. Both mutations significantly reduced the *PLP1/DM20* ratio, to less than 20% of normal, in the transfection experiments. Additionally, we analyzed RNA from patient fibroblasts, and found a reduction of the *PLP1 /DM20* ratio, but no aberrant splice forms. While the +7A>G mutation is just adjacent to the canonical donor site, the deep intronic point mutation is uniquely able to influence the choice of donor sites at a considerable distance, and may represent a new class of elements, FRISE, Far-Reaching Intronic Splicing Enhancers.

Integrating and visualizing human genome in a multi-Scale three dimensional model. *W. J. Zheng, T. M. Asbury*
Biostat, Bioinfo & Epi, Medical University of South Carolina, Charleston, SC.

A significant portion of genomic data that is currently being generated, such as histone modifications, extends beyond traditional primary sequence information. Nevertheless, genome browsers, such as the USCS Genome Database Browser, are specifically aimed at viewing primary sequence information. Although supplemental information can easily be annotated via new tracks, representing structural hierarchies and interactions is quite difficult, particularly across non-contiguous genomic segments. We present a multi-scale visualization framework to examine integrated epigenomic data within a three-dimensional model of the human genome. Our physical genome model is built using an object-oriented, hierarchical architecture and incorporates experimental data from atomic to nuclear scales. At the atomic level, we have modeled the human genome sequence with the atomic structure of each base. Genome-wide nucleosome positioning data were used from a recent high-throughput technique combining MNase digestion and ChIP-sequencing. The lower resolutions of our model, i.e., the 30nm chromatin and nuclear scales, contain data that are statistically derived. The model can provide unique insights into the interplay of various sources of genomic information. The complete model is a hybrid of rich detail in the atomic domain and progressively more statistically-based information in lower resolutions. The model is highly flexible in its ability to incorporate new data from different scales, while ensuring consistency at all levels. We view our model as a platform capable of integrating present and future data at the genomic scale. As more data becomes available, improved models should not only be able to provide the researchers with a foundation for genome visualization, but also with a comprehensive analytical platform to investigate sequential, structural and spatial details of the genome.

A Haplotype-free Approach for Cross Platform Imputation. *P. Lin, J. P. Rice* Washington University in St. Louis, St. Louis, MO.

For genome-wide association study, combining new samples with existing samples can increase power. The challenge to combine cross-platform samples is that different platforms have different choices of SNPs. One way to solve the problem is to impute unknown SNPs from known SNPs. Traditional methods for SNP imputation rely heavily on haplotype information from HapMap Project. However, haplotype information is not always available. Here, we propose a haplotype-free approach. Based on 423 individuals both genotyped by Illumina 500K chips and Affymetrix 500K chips, we predict SNPs in one platform by using logistical regression to forward select predictor SNPs among surrounding SNPs in the other platform. We demonstrate that our method has average concordance rate 97.6% (median 99.3%) on all SNPs of chr22, predicting from Illumina 500K chips to Affymetrix 500K chips, and 93.6% (median 99.1%) from Affymetrix to Illumina. The result is comparable to the program IMPUTE with average concordance rate 96.5% (median 99.2%) from Illumina 500K to Affymetrix, and 90.5% (median 95.2%) from Affymetrix to Illumina on the same data. In addition, we demonstrate that our method can predict better for more than half of SNPs. Our method is well calibrated in that the predicted probability is consistent with the observational probability distribution. We introduce a new statistic that quantifies the agreement between observed and inputed genotypes. This statistic can be used to assess the quality of the imputation (i.e., not all SNPs are amenable to imputation), and can be used to contrast different imputation methods.

Increased risk of monoclonal gammopathy of undetermined significance (MGUS) and lymphoid tumors among first-degree relatives of MGUS cases. *L. R. Goldin[1], S. Y. Kristinsson[2], N. E. Caporaso[1], M. Bjorkholm[2], I. Turesson[3], O. Landgren[1]* 1) Genetic Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, Bethesda, MD. USA; 2) Dept. of Medicine, Hematology Ctr, Karolinska U. Hospital, Stockholm, Sweden; 3) Department of Medicine, Section of Hematology, Malmo University Hospital, Malmo, Sweden.

MGUS is a generally asymptomatic plasma-cell disorder with an elevated monoclonal immunoglobulin of less than 3 g/dl in the absence of a lymphoproliferative (LP) malignancy. MGUS is a precursor to multiple myeloma (MM) and other lymphoid tumors, transforming at the rate of approximately 1% per yr. Genetic factors have been shown to be important for MM and other LP tumors but the genetic relationship to the precursor trait is not known. We identified 4488 MGUS cases diagnosed in major hematology outpatient units in Sweden (1967-2005) with linkable relatives. Using the population-based Multigenerational Registry, we obtained 17,628 controls and first-degree relatives of cases(n=14689) and controls (n=58698). Relatives were linked with hospital outpatient registries and the Cancer Registry to define occurrence of MGUS and other LP tumors. We applied a marginal survival model with a sandwich covariance estimator to take into account familial dependencies. Relatives of MGUS cases were at significantly increased risk for MGUS (HR=2.84,1.45-5.57), MM (HR=2.87,1.92-4.27), Waldenstrom macroglobulinemia (HR=4.94,1.32-18.46), and chronic lymphocytic leukemia (CLL) (HR=2.05,1.22-3.43) but not for other lymphomas. Thus, shared genes likely contribute to risk of MGUS and related LP tumors, MGUS being an early genetic lesion in the pathway to malignancy. Our candidate gene studies show possible associations of LP tumors with apoptosis genes.

ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) IN A GIRL LATER DIAGNOSED WITH CARDIO-FACIO-CUTANEOUS SYNDROME. *C. Vinkler[1,2], M. Michelson-Kerman[1,2], M. Yanoov-Sharav[1,2], T. Lerman-Sagie[2,3], D. Lev[1,2]* 1) Inst Medical Genetics, Wolfson Medical Ctr, Holon, Israel; 2) Metabolic Neurogenetic Clinic,Wolfson Medical Ctr, Holon, Israel; 3) pediatric Neurology Unit, Wolfson Medical Center, Holon. Israel.

Cardio-facio-cutaneous syndrome (CFC) is characterized by a heart defect, facial dysmormphism, and ectodermal abnormalities as well as mental retardation. Clinically it overlaps with Noonan syndrome and Costello syndrome which are caused by mutations in the proto-oncogenes PTPN11 and HRAS as well as other genes. These genes encode molecules in the RAS/RAF/MEK/ERK signaling pathway. Tumors were reported both in Noonan Syndrome and Costello syndrome. Malignancies were rarely reported in CFC. Two CFC patients have been previously reported with ALL and were associated with mutation in the BRAF gene. We report a case of ALL in an 18 months old girl who was later diagnosed with CFC. This 3 years old girl was followed in our clinic since her birth because of dysmorphic features and significant motor and language delay. She had sparse curly hair, prominent forehead, coarse facial features, hypertelorism, earlobe creases, bulbous nose, tented mouth, broad nasal bridge, hyperkeratosis in her hands and feet and hypermobility of the knees and hips joins. No cardiac involvement was identified yet. At the age of 3 years she did not walk or gain expressive language. The combination of ALL at the age of 18 months and the distinct clinical findings including her facial appearance as well as her ectodermal findings and developmental delay, support the diagnosis of CFC syndrome. Mutations screening in the BRAF, HRAS, KRAS, MEK1/2 and RAF-1 gene are currently ongoing. The risk of malignancies and type of tumors are different between syndromes of the RAS/RAF/MEK/ERK pathway. Very few patients were described with malignancies thus far. The two patients previously described with CFC and ALL carried a mutation in the BRAF gene. It is suggested that careful monitoring for malignancies should be considered as part of the regular management of CFC.

Identification of a novel missense mutation at an invariant position of the PROKR2 gene in a patient with Kallmann syndrome. *E. M. SHAH[1, 2], H. G. KIM[1, 2], L. P. CHORICH[1, 2], L. C. LAYMAN[1, 2]* 1) Dept. of OB/GYN, Section of Reproductive Endocrinology, Infertility & Genetics, The Medical College of Georgia Augusta, GA 30912; 2) Reproductive Medicine Program, Developmental Neurobiology Program, Neuroscience Program, Institute of Molecular Medicine & Genetics, The Medical College of Georgia Augusta,GA 30912.

Idiopathic hypogonadotropic hypogonadism (IHH) is a developmental disorder characterized by a derangement in hypothalamic gonadotropin releasing hormone (GnRH) secretion or action. Kallmann syndrome (KS) combines IHH with anosmia and results from impaired embryologic GnRH and olfactory neuron migration. Patients with IHH/KS present with absent or impaired pubertal development, low serum sex steroids, and low serum gonadotropin levels. Although mutations in the FGFR1, KAL1, and GNRHR genes have been reported in ~20-30% of IHH/KS patients, the molecular basis of most patients remains unknown. Heterozygous, homozygous, and compound heterozygous loss-of-function mutations in the gene encoding the prokineticin receptor 2 (PROKR2) have been reported recently in patients with IHH/KS. We wanted to determine the prevalence of PROKR2 mutations in our sample of patients with IHH/KS. To date, PCR and DNA sequencing have been performed on 53 IHH/KS (26 anosmic, 6 hyposmic, and 21 normosmic) patients using primers designed to amplify both protein encoding exons. DNA sequence was analyzed using the CodonCode Aligner program. No gene deletions were identified in any IHH/KS patient, but 1/53 (1.9%) patients had a heterozygous missense mutation (Arg135Cys) not reported in the SNP database. This novel missense mutation interrupts a highly conserved, invariant amino acid within the first extracellular loop (including amino acid residues 111-136) near the junction of the third transmembrane domain. Our findings indicate that mutations in the PROKR2 gene may be responsible for ~2% of KS patients, but additional normosmic IHH and KS patients need to be studied.

Association between IL17RD gene and Crohns disease and gene-gene interaction in the IL23-IL17 pathway. *L. Mei[1], K. Taylor[1], D. McGovern[2], S. Targan[2], J. Rotter[1]* 1) Medical Genetics, Cedars-Sinai Medical Center, Los Angeles, CA; 2) Inflammatory Bowel Disease Center, Cedars-Sinai Medical Center, Los Angeles, CA.

Accumulating evidence has shown that the IL23-IL17 pathway is important in pathogenesis of Crohns disease (CD). We, and others, have shown that IL23-IL17 pathway genes including IL12b, IL12RB1, IL12RB2, IL17A, IL17RA are associated with CD. IL17RD, another member of IL17 receptor family, has been detected in various cells, but its role in human CD is not clear. The aim of our study was to determine whether IL17RD is associated with CD and whether there is a gene-gene interaction within IL23-IL17 pathway genes. Methods: 763 CD subjects and 254 controls were genotyped for single nucleotide polymorphisms in the IL23A, IL23R, IL17A, IL17RA, IL12B, IL12RB1, IL12RB2 and IL17RD genes using Illumina and ABI platforms. Haplotypes were assigned using Phase v2 and were tested for association with CD by chi square test. We utilized multidimensionality reduction (MDR) to explore gene-gene interactions. Results: Two Blocks (B) of IL17RD were associated with CD. CD patients had a higher frequency of haplotype2 in block2 (B2H2, 55.0% vs. 45.4%, OR=1.5, p=0.01) and a lower frequency of B1H2 (39.1% vs. 50.2%, OR=0.64, p=0.002) and B2H3 (37.8% vs. 47.4%, OR=0.68, p=0.01) when compared with controls. The results of other pathway genes have been previously reported by our group. Haplotypes with increased risk for CD were observed in the IL23R_ B2H1 and B3H1, IL17A_H2, IL17RA_B2H4, IL12RB1_H1 and IL12RB2_H3; haplotypes with decreased risk were observed in the IL23R_B2H2 and B3H2, IL17A_H4, IL17RA_B1H3, IL12B_H1 and IL12RB2_H4. MDR analysis suggested interaction between IL23R_B2H2, IL12RB2_H4 and IL17RD_B2H2 (CV consistency 10/10, tested accuracy 59.7%, p=0.002). The following logistic regression analysis confirmed the interaction (IL23R_B2H2*IL12RB2_H4, p<0.0001; IL23R_B2H2*IL17RD_B2H2, p=0.02). Conclusion: IL17RD is significantly associated with CD and is likely to interact with IL23R in the risk of developing CD. Gene-gene interactions in the IL23-IL17 pathway may play an important role in the pathophysiologic mechanisms of the disease.

Identifying epigenetic patterns that are associated with atherosclerosis. *J. J. Connelly[1], C. Markunas[1], D. R. Crosslin[1], D. Biscocho[1], S. Gadson[1], J. F. Doss[1], T. S. Furey[1], T. Wang[1], S. Nelson[1], P. D. Ellis[2], C. F. Langford[2], P. J. Goldschmidt-Clermont[3], D. Seo[3], S. H. Shah[1], W. E. Kraus[1], E. R. Hauser[1], S. G. Gregory[1]* 1) Duke University, Durham, NC; 2) The Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK; 3) University of Miami, Miami, FL.

DNA methylation plays a role in the transition of smooth muscle cells (SMCs) from a contractile, differentiated state to a migratory, proliferative, anti-apoptotic state. These etiological changes lead to atherosclerotic lesion formation and coronary artery disease (CAD). Passaging aortic SMCs in culture results in similar characteristics of migratory SMCs and differentially methylated loci can be detected in early versus late passages (*ESR1*, *ESR2*, *MCT3*). The same changes have also been detected in atherosclerotic lesions. These data suggest that changes in DNA methylation status of genes of proliferative SMCs could play a role in CAD initiation and progression. To identify these epigenetically regulated candidate genes we investigated the DNA methylation status of passaged aortic SMCs by comparative methylation hybridization using genomic tile path arrays. We identified 346 significantly differentially methylated regions in aortic SMCs at passage 5 versus passage 8 (p0.01). One of these regions contains a gene, collagen type XV, alpha 1 (*COL15A1*) that is hypomethylated, upregulated with passage (p=0.013), and responsive to decitabine treatment (p=0.003). SMCs secrete COL15A1, a molecule known to interact with LDL and to be enriched in plaques. We hypothesized that *COL15A1*, its expression level and its methylation status may play a role in atherosclerosis. We show that *COL15A1* gene expression is upregulated in diseased versus non-diseased aorta (p=0.02) and that a specific intronic polymorphism, rs4142986, within *COL15A1* leads to a dose dependant decrease in transcription of the gene in aorta samples (p=0.008). This G/C polymorphism is the only SNP in the linkage disequilibrium bin (r2 cutoff=0.7) and creates a CpG site within a region of putative differential methylation. We present this non-biased method as a novel cellular based approach to defining and characterizing polymorphisms associated with CAD.

First trimester sequential screen results which indicate a moderately increased risk of Down syndrome (1/50 - 1/270): How do patients respond to these results? *D. Wagner[1], C. Pargas[2], A. Donnenfeld[1]* 1) Genzyme Genetics, Philadelphia, PA; 2) Genzyme Genetics, Sante Fe, NM.

Background: The Genzyme Genetics first trimester sequential screening (SS1) program utilizes a Down syndrome (DS) risk cut-off of 1/50 as a screen positive result. This will detect 70% of DS pregnancies at a 1.2% screen positive rate. Following completion of the second trimester sequential screen (SS2), 90% of DS fetuses will be detected utilizing a risk cut-off of 1/270. **Objective:** To document patient decisions after being informed their SS1 results were within the normal range according to our protocol, but revealed a moderately increased risk of DS between 1/50-1/270. **Methods:** A query of our sequential screening database was performed on patients in the Philadelphia/South Jersey area seen from January, 2006-March, 2008. All patients with SS1 results in the 1/50-1/270 range were identified. Patient decisions regarding invasive testing (prior to completing SS2), completing the SS2 blood draw, or no additional testing were tabulated. Patients who had a spontaneous loss, a family history indication for a diagnostic procedure, an abnormal ultrasound, or a positive Trisomy 18 screen were excluded from the analysis. **Results:** A total of 10,850 patients underwent SS1 testing during this interval. 557 patients (5.1%) had risks between 1/50 - 1/270.

Total # of pts	Pts who had SS2	CVS	Amnio before SS2	No further tests
557	515 (93%)	5 (0.9%)	13 (2.3%)	24 (4.3%)

Conclusion: The majority (93%) of patients in the intermediate DS risk range completed the sequential screening process before making any decisions regarding invasive testing. Only 3.2 % of these patients and 0.16% of all patients decided to pursue a CVS or amniocentesis without completing the SS2 blood draw. Using a 1/50 risk cut-off in the first trimester is an effective screening policy for sequential screening.

Cystinosis: Novel Dental Findings Involving Enamel and Root Anomalies. *C. W. Bassim[1], D. L. Domingo[1], J. Z. Balog[2], JP. Guadagnini[1], W. A. Gahl[2], T. C. Hart[1]* 1) NIDCR, NIH, Bethesda, MD; 2) NHGRI, NIH, Bethesda, MD.

OBJECTIVE: Cystinosis is an autosomal recessive lysosomal storage disorder caused by loss-of-function mutations of the cystinosin gene (CTNS), leading to cellular damage from cystine accumulation. Clinical features include growth retardation, renal failure, and hypophosphatemic rickets. Oral manifestations remain poorly defined. This study characterized dental anomalies of enamel and root morphology, both derived from enamel epithelium, in cystinosis patients and unaffected controls. **METHODS:** Oral clinical and radiographic evaluations were performed on 44 nephropathic cystinosis patients (22 males, 22 females; mean age=17.21.0, range 10-41 years) and 93 healthy controls (36 males, 57 females, mean age=18.00.4, range=10-25 years). The Taurodontism Index (TI), measuring the size of the pulp chamber in multi-rooted teeth, was used to evaluate mature first and second molar pulp chamber size from radiographs. The Developmental Defects of Enamel (DDE) Index was used to determine the prevalence of enamel defects from clinical photographs of teeth, i.e., 104 maxillary incisors each from cystinosis patients and age- and gender- matched controls. **RESULTS:** All three classifications of taurodontism were significantly more prevalent in cystinosis patients than in controls, indicating increasing severities of pulpal enlargement. Hypotaurodontism was present in 71/252 molars (28%) vs. 114/652 molars (17%; $p<0.0001$); mesotaurodontism in 11/252 molars (4%) vs. 4/652 molars (1%; $p<0.0001$); and hypertaurodontism in 5/252 molars (2%) vs. 2/652 molars (0%; $p=0.020$). For every unit increase in TI Index, the odds of increasing pulpal enlargement being associated with cystinosis status increased by 8%, even when adjusted for age (OR=1.08, $p<0.0001$). Enamel defects (diffuse opacities or hypoplasia) were found in 42% (44/104 incisors) of the cystinosis group, significantly more than for controls (1/104 incisors, $p<0.0001$). **CONCLUSIONS:** Our findings indicate novel dental features in cystinosis. Cystinosis associated variations in tooth morphology may be part of the disease spectrum affecting mineralized tissues and may shed additional insight on this complex disease.

Living with Costello Syndrome: Quality of Life Issues in Older Individuals. *E. G. Hopkins[1], A. E. Lin[2], K. E. Krepkovich[3], L. Nicholson[1], M. E. Axelrad[4], K. Sol-Church[5], J. Hossain[6], K. W. Gripp[1]* 1) Medical Genetics, AI duPont Hospital, Wilmington, DE; 2) Genetics and Teratology Unit, MGH for Children, Boston, MA; 3) Center for Human Genetics, BMC, Boston, MA; 4) Texas Childrens, Child Psychology, BCM, Houston, TX; 5) Biomedical Research, Nemours Childrens Clinic, Wilmington, DE; 6) Nemours, Research Programs, Wilmington, DE.

Costello syndrome (CS), a rare condition due to mutations in the *HRAS* gene, consists of coarse facial features, mental retardation, cardiac abnormalities, musculoskeletal anomalies, and an increased incidence of neoplasia. We designed a two part Quality of Life (QoL) survey to obtain (1) objective information from caregivers regarding daily living skills and activities, and (2) subjective information from older individuals with CS assessing self-esteem, life satisfaction and interpersonal relations. Questionnaires were collected from 13 caregivers and 11 individuals with CS. RESULTS: Data was analyzed to describe day-to-day life, as well as potential impediments on QoL for older individuals. A Wilcoxon signed rank test demonstrated significant difference between the QoL total scores based on caregiver report and the QoL total scores based on self-report ($p<.008$). A Spearmans rho revealed an inverse relationship between caregiver QoL total scores and the number of major medical issues experienced by the individual with CS ($N = -.549$) and minimal correlation between QoL total scores based on self-report and the number of major medical issues ($N = -.107$). CONCLUSIONS: Four impediments to QoL for CS individuals include desire for close relationships, lack of independence, plans for the future, and medical issues. As the number of major medical issues an individual experiences increases, the QoL score determined by caregiver report decreases; however, there is little effect on QoL score based on self-report. Recommendations to maximize QoL include encouraging autonomy without compromising safety, discussing relationships and future plans, and continuing to practice preventative medicine. Genetic counselors must be aware of factors affecting QoL to provide appropriate psychosocial counseling and anticipatory guidance.

Population demographic history causes the appearance of recombination hotspots. *H. R. Johnston[1], D. J. Cutler[2]*
1) Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD; 2) Department of Human Genetics, Emory University School of Medicine, Atlanta, GA.

The existence of human recombination hotspots is widely accepted. However, the vast majority of human hotspots have been identified only through inferences based on Linkage Disequilibrium (LD). These inferences are made using a model of human history with a constant effective population size. Relaxing this assumption, we show that models of human history with large ancestral populations, followed by long, shallow bottlenecks, and culminating in rapid population growth produce variation and LD patterns very similar to humans. When recombination rates are estimated on data produced by this model, LDHat infers tens of thousands of recombination hotspots, with ~50% of all recombination occurring in ~10% of the genome. This model gives the appearance of recombination hotspots due to variation in the time to most recent common ancestor. Apparent recombination hotspots are regions of the genome that are much older than their neighbors.

Our demographic model can be distinguished from true recombination rate variation by several predictions. First, our model predicts substantial variation in SNP density across the genome, and apparent hotspots ought to be associated with higher SNP density. There are 4.1 SNPs per 1,000 bases in hotspots, and 3.5 SNPs per 1000 bases elsewhere, as predicted. Second, the average recombination rate across all hotspots can be estimated from the correlation in SNP density on either side of the hotspot. Apparent hotspots have an average recombination rate of 1 cM/Mb, the genome-wide average. Finally, we reexamine published data by Coop, et al. that mapped recombination events in pedigrees. The hotspot model predicts that the center of windows containing known recombinations ought to be enriched for hotspots, while our model predicts that the edges of the recombination windows should show enrichment for apparent hotspots because apparent hotspots are rich in informative SNPs. We find that the centers of recombination windows have exactly the genome-wide average of apparent hotspots, while the edges are enriched by ~50%.

PREST-plus: detection of pedigree error and cryptic relatedness among individuals that allows for high-throughput genotype data and adjusts for large-scale multiple hypothesis testing. *L. Sun*[1,2,3], *T. Chiang*[4] 1) Dept Public Health Sci; 2) Dept Statistics, Univ of Toronto; 3) Genetics and Genomic Biology, Sickkids; 4) Centre for Computational Biology, Sickkids, Toronto, Canada.

Genetic studies collect both family and population data, and most statistical mapping methods are not robust to pedigree errors nor cryptic relatedness (e.g. Boehnke and Cox, 1997; Thornton and McPeek, 2007). However, pedigree errors are well documented in linkage analyses and more recently cryptic relatedness in association studies. PREST (Pedigree RElationship Statistical Test) is a C program for detection of pedigree errors by use of genetic marker data. PREST implements the EIBD, AIBS, IBS, and MLLR test statistics, as well as an EM-based estimator of IBD sharing by two individuals developed by McPeek and Sun (2000) and Sun et al. (2002). PREST has been shown to be an effective tool in identifying misspecified relationships using genome-wide (GW) linkage marker data (e.g. Keenan et al, 2008; Samudrala et al, 2008). However, a number of statistical and computational issues remain, and new challenges emerge as the ability for high-density genotyping increases. PREST-plus aims to increase the efficiency and interface of PREST. For example, to deal with the computational load of the MLLR test which requires simulation to assess significance, PREST-plus adopts a dynamic procedure that estimates the number of simulation replicates needed for each test, replacing the 2-stage screening procedure used by PREST. The computational saving is more than 10-fold. PREST-plus also allows users to specify a number of options in a script file including checking for errors across pedigrees. In addition, a R program is developed for post-PREST analyses that incorporates the False Discovery Rate (FDR) methodology (Benjamini and Hochberg, 1995) to adjust for the large-scale multiple hypothesis testing, as well as the Stratified FDR method (Sun et al. 2006) to improve power by utilizing the knowledge that the expected error rates are different for different types of putative relationship (e.g. full-sib vs. unrelated). We also present results of application of PREST-plus to existing GW linkage and association studies.

Sequence determinants of human microsatellite variability. *T. J. Pemberton, C. I. Sandefur, M. Jakobsson, N. A. Rosenberg* Department of Human Genetics, University of Michigan, Ann Arbor, MI.

Microsatellite loci consist of short tandem repeats (STR) that vary in length between individuals and that generally have many distinct alleles within the human population. The high level of variability for microsatellites compared to other regions in the genome has led to their use as markers in linkage analysis and population-genetic studies. In this study we have used genotypes at 631 microsatellite loci in 1048 worldwide individuals from the HGDP-CEPH cell line panel to investigate the effect of sequence properties of microsatellites on their level of variability. We consider an STR to be a repeat unit of 2-5 nucleotides with four or more consecutive repeats; a microsatellite locus may contain one or more STR regions embedded between the PCR primers used to amplify the locus. The number of STRs in the microsatellite sequence, the sequences of their repeat units, and the numbers of repeats, were identified for each microsatellite from the DNA sequence in the human RefSeq database extracted using its PCR primer pair. Calibrating PCR fragment lengths in individual genotypes by using the RefSeq sequence enabled us to infer repeat number in the HGDP dataset and to calculate the mean number of repeats (as opposed to the mean PCR fragment length), under the assumption that differences in PCR fragment length are due to differences in the numbers of repeats in the embedded STRs. We find that the sequence of the repeat unit is an important factor in predicting microsatellite heterozygosity (e.g. $P = 6.01 \times 10^{-4}$ for tetranucleotide microsatellites with one embedded STR with repeat unit AAAT versus those with repeat unit AAGG, Wilcoxon test). The mean number of repeats is observed to be positively correlated with heterozygosity (e.g. Spearman's = 0.564 for dinucleotide microsatellites with one embedded STR, $P = 1.16 \times 10^{-3}$). Finally, we find that the number of STRs in a microsatellite's sequence increases its heterozygosity (e.g. $P = 2.69 \times 10^{-5}$ for tetranucleotide microsatellites with one STR versus those with two STRs, Wilcoxon test). These results further our knowledge of the sequence determinants of microsatellite variability.

/Foxl2/ functions in sex determination and histogenesis throughout mouse ovary development. *E. Pelosi[1], J. E. Garcia-Ortiz[1,2], S. Omari[1], T. Nedorezov[1], Y. Piao[1], J. Karmazin[1], M. Uda[3], A. Cao[3], A. Forabosco[4], D. Schlessinger[1], C. Ottolenghi[1]* 1) Laboratory of Genetics, NIA/NIH-IRP, Baltimore, USA; 2) División de Genética, Centro de Investigación Biomédica de Occidente, CMNO-IMSS, Guadalajara, México; 3) Istituto di Neurogenetica e Neurofarmacologia, Consiglio Nazionale delle Ricerche, Cittadella Universitaria di Monserrato, Monserrato, Cagliari, Italy; 4) Unità di Genetica Medica, Università di Modena, Italy.

Partial loss of function of the transcription factor /FOXL2/ leads to premature ovarian failure in women. In goats and mice, /Foxl2/ is required for maintenance, and possibly induction, of female sex determination. We have thus suggested that in all mammals, female sex determining gene(s) in the /Foxl2/ pathway may operate throughout development and postnatal life, both to mature the fetal ovary and to maintain female reproductive function. Here we investigated this possibility by expression profiling of mouse ovaries that lack /Foxl2/ or /Foxl2/ and /Wnt4/ (a second gene critical for gonadal development). Compared to wild-type, loss of one copy of /Foxl2/ showed strong gene dosage for a subset of ovarian developmental genes; and /Foxl2/-null ovaries were depleted of follicle formation markers and enriched for a number of testis tubule markers. Consistent with early anti-testis and pro-ovary actions, a /Foxl2/ transgene reduced the levels of key testis markers, including /Sox9/, in embryonic XY gonads, and increased the levels of ovarian somatic and meiotic factors, e.g. /Fst /and /Sycp3/, /in XX littermates. We used in silico approaches to identify novel candidate anti-testis genes and to infer that the mouse transcriptome progressively converges toward a testis-like signature, already notable at birth, in /Foxl2/- and /Wnt4/-null ovaries. These data 1) support the proposal of anti-testis, continued function of /Foxl2/ from the earliest steps of ovary differentiation to adult; and 2) identify additional candidate genes associated with sex determination and ovary histogenesis.

Duplication restricted to ICR2 (*KCNQ1, KCNQ1OT1* and *CDKN1C* genes) at 11p15 segregating in a family with five children affected by Silver-Russell syndrome. *A. M. Vianna-Morgante[1], A. Bonaldi[1], R. S. Honjo[2], D. R. Bertola[2], L. M. J. Albano[2], I. M. Furquim[2], C. A. Kim[2], J. F. Mazzeu[1]* 1) Departamento de Genética e Biologia Evolutiva, Instituto de Biociências, Universidade de São Paulo, São Paulo, Brazil; 2) Unidade de Genética, Instituto da Criança, Hospital das Clínicas, Universidade de São Paulo, São Paulo, Brazil.

Silver-Russell syndrome (SRS, MIM180860) is a clinically and genetically heterogeneous syndrome characterized mainly by severe intrauterine and postnatal growth retardation, craniofacial features including frontal bossing and small triangular face with pointed chin, and body asymmetry. Imprinted regions on chromosomes 7 and 11 have been implicated in the aetiology of SRS: while maternal uniparental disomy of chromosome 7 has been identified in about 10% of patients, methylation defects at 11p15 have been described as a major cause of SRS, being present in 35-65% of patients. Hypomethylation on the telomeric imprinting center on chromosome 11 (ICR1) is the most frequent alteration, and a few maternal duplications of the segment including genes regulated by ICR1 have also been described. A single maternal duplication that encompasses ICR2, but not ICR1, has been reported. We found a similar duplication segregating in a family with five children affected by SRS, two boys and three girls born to two clinically normal sisters. The analysis of the ICR regions on 11p15 (*H19*, *IGF2*, *KCNQ1*, *KCNQ1OT1* and *CDKN1C* genes) was carried out by MS-MLPA (MRC-Holland). The duplicated region was restricted to the centromeric ICR2, and encompassed the *KCNQ1*, *KCNQ1OT1* and *CDKN1C* genes. This duplication was present in the five children affected by SRS, in their normal mothers and in a normal maternal uncle, but not in the maternal grandmother, indicating that the deceased maternal grandfather was the carrier. An aunt, an uncle and a sister of the SRS children, all clinically normal, did not carry the duplication. This family provides further evidence that ICR2 on 11p15 is involved in the etiology of SRS. In addition, it shows that duplication of the *KCNQ1*, *KCNQ1OT1* and *CDKN1C* genes does not have clinical effects when paternally transmitted.

Association study of phencyclidine-responsive genes with schizophrenia. *X. Deng[1], H. Shibata[1], T. Kuroki[2], T. Nakahara[2], K. Hashimoto[2], H. Ninomiya[3], N. Iwata[4], N. Ozaki[5], Y. Fukumaki[1]* 1) Div. Hum. Mole. Genet., Res. Ctr. Genet. Info., Med. Inst. Bioreg., Kyushu Univ., Japan; 2) National Hospital Organization Hizen Psych. Ctr., Japan; 3) Dazaifu Hospital, Japan; 4) Dept. Psych., Fujita Health Univ.,Japan; 5) Dept. Neuropsych., Nagoya Univ., Japan.

Since schizophrenia-like symptoms are produced by administration of phencyclidine (PCP), a noncompetitive antagonist of N-metyl-D-aspartate (NMDA) receptors, PCP-responsive genes could be involved in pathophysiology of schizophrenia. We have injected PCP (5mg/kg) to two groups of twelve Wistar rats and isolated 5 different parts of the brain of rats one hour and four hours, respectively, after the injection. The same number of rats treated with saline was also prepared as controls. We compared gene expression profiles of these tissues using AGILENT microarrays. Ninety annotated genes and 21 ESTs showed altered expression after the treatment. We have successfully identified ten genes of which expression was altered at least two folds after quantitative evaluation by real-time PCR. The PCP-responsive genes were subjected to the locus-wide association study to identify susceptibility genes for schizophrenia. To date six of them have been analyzed by the case-control association procedure. In single marker analysis, there was no association of SNPs of these genes with schizophrenia. However, in haplotype analysis, significant associations were detected in combinations of two SNPs of *BTG2* and *PLAT*($P = 1.4\times10^{-6}$, $P = 1\times10^{-3}$). These associations were still significant even after correction of multiple testing by False Discovery Rate. These results indicate that at least one susceptibility locus for schizophrenia may be located within or very close to the *BTG2* and *PLAT* regions in the Japanese population.

Characterization of Circulating Tumor Cells by Fluorescence In-Situ Hybridization. *J. F. Swennenhuis[2], A. G. J. Tibbe[2], R. Levink[2], R. C. J. Sipkema[2], L. W. M. M. Terstappen[1]* 1) Twente University, Enschede, Netherlands; 2) Immunicon Europe, Enschede, Netherlands.

Background: Tumor cells in blood of patients with metastatic carcinomas have been associated with poor survival prospects. Further characterization of these cells may provide further insights into the metastatic process. In this study we demonstrate feasibility of reproducible analysis of Fluorescent in situ hybridization (FISH) of CTC in patients with metastatic prostate cancer. Methods: CTC were enumerated in 7.5 mL of blood with the CellSearch system. After enumeration of Cytokeratin+, CD45-, nucleated cells, the cells are fixed in their original position and the fluid from the analysis cartridge is removed. Cartridges were hybridized with probes detecting chromosome 1, 7, 8 and 17 and placed on a fluorescent microscope. The previous identified CTCs are revisited and fluorescent images of the probes were acquired. Leukocytes surrounding the CTC were used as internal controls. Results: FISH was applied to 199 CTC containing blood samples from 70 metastatic prostate cancer patients. The 199 samples contained a total of 23,341 CTC (mean=11, average= 116, SD= 570). Of the 23,341 CTC, 6,068 (26%) were lost during the fixation and FISH. Of the remaining 17,273 CTC no FISH signals were detected in 51% of the CTC that were undergoing apoptosis. The 8,809 (49%) evaluable CTC contained on average 2.8 copies of chromosome 1, 2.7 copies of chromosome 7, 3.1 copies of chromosome 8 and 2.3 copies of chromosome 17. 80% of the CTC were aneuploid. In only 6 patients no aneuploid cells were detected and these patients only had 1 or 2 CTC. Heterogeneity in the chromosomal abnormalities was observed between CTC of different patients as well as among CTC of the same patient. Conclusions: Cytogenetic composition of CTC can be assessed after they have been identified by the CellSearch system. The relation between the presence of aneusomy, the extent of amplification and outcome can now be investigated.

Whole Genome Scan in the CARRIAGE Family Study: Evidence of Novel Quantitative Trait Loci (QTL) for Osteoarthritis Trait on Chromosome 8. *HC. Chen[1,4], SH. Shah[2,3], YJ. Li[2], S. Nelson[2], C. Haynes[2], J. Johnson[2], T. Stabler[1], ER. Hauser[2], SS. Gregory[2], WE. Kraus[3], VB. Kraus[1,4]* 1) Division of Rheumatology; 2) Center for Human Genetics; 3) Division of Cardiology; 4) Department of Pathology, Duke University Medical Center.

Background: Osteoarthritis (OA) is a multifactorial disorder associated with various risk factors. Recently, the genetic contribution to OA has been recognized. OA-related biomarkers have been proposed as sensitive and reliable markers to quantify OA burden. Therefore, finding genes associated with variation in OA-related biomarkers may provide distinctive insights into genetic determinants of OA. Methods: The extended CARRIAGE family consists of 3357 pedigreed members in the US. Ascertainment of 365 members was accomplished. We measured five OA-related biomarkers: HA (hyaluronan), COMP (cartilage oligomeric matrix protein), PIIANP (type IIA collagen N-propeptide), CPII (type II procollagen carboxy-propeptide), and C2C (neoepitope from cleavage of CII). Genotyping was performed using Illumina BeadChip with 6,090 SNP markers. Variance components as implemented in SOLAR were used to estimate heritabilities of quantitative traits, and probabilities of IBD using a polygenic model. Results: Four of five biomarkers showed significant heritability [PIIANP (h2r=0.57), HA (h2r=0.49), COMP (h2r=0.43), $p< 0.005$ for each; C2C (h2r=0.30), p=0.01] after adjusting for age and sex. The maximum multipoint LOD score of 3.90 was achieved for PIIANP on chromosome 8p23 (1-16 cM). For COMP, the strongest evidence of linkage was also on chromosome 8 (LOD=3.2, 61-69 cM and LOD=2.5, 146-153 cM). For HA and C2C, the maximum QTL were identified on chromosome 6q16 and 5q31, respectively. Additional QTL (LOD >2.0) were observed on chromosome 9, 13 and 15 for PIIANP and chromosome 14 for COMP. Conclusions: We report the first evidence of genetic contribution to OA-related biomarkers, identified in one extended family. We identified several new genetic loci for OA-related biomarkers. Further studies of the candidate genes at these loci may provide new insights for the mechanisms of cartilage metabolism, development and progression of OA.

Colorectal Cancer and *XRCC2*: A Three-center Meta Analysis. *K. Curtin[1], W.-Y. Lin[2], R. George[2], M. Katory[2], J. Shorto[2], D. T. Bishop[3], A. Cox[2], N. J. Camp[1]* 1) Genetic Epidemiology, University of Utah School of Medicine, Salt Lake City, UT, USA; 2) Inst. for Cancer Studies, Sheffield School of Medicine, UK; 3) Epidemiology and Biostatistics, Leeds Inst. of Molecular Medicine, UK.

Double-stranded DNA repair polymorphisms may play a role in cancer etiology. Associations of 17 SNPs in *XRCC2* and colorectal cancer (CRC) were investigated in a three center meta study including two UK case-control cohorts (Sheffield and Leeds) and family-based cases in high-risk Utah pedigrees and matched controls (total: 1132 cases, 1092 controls). The SNPs studied were 15 tagging-SNPs selected from an analysis using HapMap/NIEHS data. In addition, two novel variants were identified from sequencing of 125 Caucasian CRC cases chosen to represent multiple CRC groups (including familial, sporadic and metastatic disease). Meta-statistics and Monte Carlo significance testing using Genie software provided valid analyses of the total resource. Similar to reports in other cancer sites, the rs3218536 R188H allele was not associated with increased risk of CRC. However, two SNPs were observed to be associated with CRC. These SNPs were in linkage disequilibrium with each other and represented only a single association finding in overall CRC (OR 1.6, 95%CI 1.1-2.4), in women (OR 2.1, 95%CI 1.2-3.7), and in rectal colon tumors (OR 2.1, 95%CI 1.3-3.4). The association was highly significant in female rectal cases (OR 3.2, 95%CI 1.5-6.7; $p<0.001$). A significant difference was found in a case-case comparison of rectal vs. proximal/distal colon cancers ($p=0.02$). Our investigation offers support for a role of *XRCC2* in susceptibility to CRC, and more specifically for rectal cancer subsite, particularly in women.

Identifying Diabetic Nephropathy Genes on Chromosome 18 in African Americans: A Dense SNP Map. *C. W. McDonough[1], M. Bostrom[2], L. Liu[3], P. J. Anderson[2], C. D. Langefeld[3], J. Divers[3], B. I. Freedman[4], D. W. Bowden[1,2,4]*
1) Program in Molecular Medicine; 2) Dept of Biochemistry; 3) Dept of Public Health Sciences; 4) Dept of Medicine; Wake Forest Univ Sch Medicine, Winston-Salem, NC USA.

Genome wide linkage scans for diabetic nephropathy (DN) in Turkish, African Americans (AA), European Americans, and American Indians have mapped a susceptibility locus to 18q21.1-23. There is also evidence that a polymorphism in the *CNDP1* gene, located on 18q, is associated with DN in Europeans and European Americans. In order to examine this region comprehensively in AAs, we performed a dense SNP map of a 17.6 Mb region: 18q21-22.2. 3,072 SNPs were genotyped in 1080 AAs with type 2 diabetes and end stage renal disease and 1080 AA healthy controls. Seventy ancestry informative markers (AIMs) were included in the SNP set for admixture adjustment. 2,816 SNPs were used for data analysis. After admixture adjustment, 233 SNPs were significantly associated with DN ($P<0.05$) under an additive and/or dominant genetic model. Seven of the top 15 associated SNPs were located within or near known genes; two were located near hypothetical genes; and six were located within intergenic regions. After prioritizing the results, three candidate genes in the region: *DCC*, *NEDD4L*, and *SERPINB7* are noteworthy. In *DCC*, deleted in colorectal carcinoma, 17 of the 152 SNPs genotyped showed evidence of association (11.2%). The most associated SNP, rs1393330, was intronic and located at the 3 end of the gene ($P=0.002$, all genetic models). Eight percent of the SNPs genotyped in *NEDD4L*, neural precursor cell expressed, developmentally down-regulated 4-like, previously associated with hypertension, were associated with DN (6/75). Four of the associated SNPs clustered in intron 1 and intron 2. rs512099, located in intron 1, showed the strongest evidence of association ($P=0.0006$, dominant model). There were five SNPs that showed evidence of association in and near (within 30kb) *SERPINB7*. The most associated SNP, rs1720843, was intronic ($P=0.005$, additive model). Overall the results are consistent with multiple variants contributing to DN. These genes warrant further investigation in order to determine their role in DN in AAs.

Assessing the prognostic utility of pharmacogenetic biomarkers. *L. Li, M. Mosteller, M. G. Ehm, M. R. Nelson*
Genetics Division, GlaxoSmithKline, Research Triangle Park, NC.

The ultimate goal of many pharmacogenetic (PGx) studies is to identify markers that may be clinically useful for differentiating clinical response and risk of adverse drug reactions (ADRs). However, a PGx marker that is statistically significant may not demonstrate prognostic or clinical utility. Summarizing and communicating the potential utility of identified markers is an important step in the biomarker development process. Our initial work focuses on markers for ADRs. We first build up a marker utility profile using well-known statistics including: odds ratio, p value, accuracy, ADR prevalence, marker prevalence, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). From these we can derive additional relevant statistics if marker screening were to be implemented: number needed to screen to avoid one ADR, relative risk of ADR, absolute reduction in ADR rate, and fraction of patients who would not receive the drug, but who could safely take it. Through exploring these statistics for ADRs with known genetic risk factors, we conclude that the strength of association and commonly used descriptors of genetic effects are insufficient to assess and communicate the usefulness of a potential biomarker. One example is *HLA-B*1502* for Carbamazepine-induced Stevens-Johnson syndrome in Asians, which has an estimated odds ratio of infinity and a discovery p value of 10^{-33}. However, we find that for every 300 patients screened, 11 would be denied treatment while one ADR would be avoided. We also investigated the relationship between predictive values, ADR prevalence () and marker prevalence (*p*) to facilitate future study design. We find that the upper bounds of PPV and NPV are determined by min[*/p, 1*] and min[*(1-)/(1-p), 1*], respectively. Given these bounds, we confirm that common variants are capable of identifying prognostically useful PGx biomarkers for relatively common ADRs. However, it is important to recognize the limited prognostic utility of common variants for rare ADRs if NPV and PPV must be close to one.

A *CFH* haplotype that tags a deletion of *CFHR1* is over represented in individuals with coronary artery disease.

J. F. Doss[1], K. S. Chen[1], J. J. Connelly[1], D. R. Crosslin[1], S. Nelson[1], T. Wang[1], D. Seo[2], J. M. Vance[2], P. J. Goldschmidt-Clermont[2], S. H. Shah[1], W. E. Kraus[1], E. R. Hauser[1], S. G. Gregory[1] 1) Duke University, Durham, NC; 2) University of Miami, Miami, FL.

Immunity and inflammation have been shown to play a major role in the development of many chronic diseases, including cardiovascular disease. Tight regulation of the complement system is necessary to prevent excessive inflammation resulting in tissue damage and heightened disease progression. Recent studies suggest that the complement system is a key component in the progression of age-related macular degeneration and atypical hemolytic uremic syndrome. Based on convergent data, we propose that impaired regulation of the complement system is also a factor in the progression of coronary artery disease (CAD). First, a proteomics study from Duke University identified lower complement factor H related 1 (CFHR1) and higher complement C3 (C3b) blood plasma levels in CAD cases versus controls (Donahue *et al*. 2006). In the complement pathway, CFHR1 acts as a cofactor in the degradation of the complement response cell targeting protein C3b. Therefore, decreased CFHR1 levels may lead to excessive C3b activity and inflammation. Second, we show that mRNA expression levels of *CFHR1* in human aorta are significantly lower in diseased versus non-diseased samples (p=0.01). Third, the region containing *CFHR1* has been shown to be copy variable. Thus, we hypothesize that *CFHR1* gene expression is altered by deletion of a segment of DNA that contains *CFHR1* and that individuals that have CAD are more likely to harbor this copy number variant (CNV). To test this, we genotyped 1920 individuals from the CATHGEN study for a common haplotype (GTGTAAAG) that tags the *CFHR1* CNV (Hughes et al 2006). We found a significant association between the haplotype and CAD in young affected individuals (p=0.001). Two SNPs within the haplotype, rs1831281 and rs2284664, were also found to be independently associated with CAD (p= 0.024 and 0.028, respectively). We are currently assessing the CNV within this region to determine if mRNA and protein levels of *CFHR1* correlate with hemizygous deletion of *CFHR1*.

Analysis of apparent homozygous runs of SNPs in genome-wide association studies. *F. J. Boehm, T. S. Lumley, K. M. Rice, B. S. Weir* Biostatistics, University of Washington, Seattle, WA.

Within the context of genome-wide association studies, some investigators have reported apparent homozygous runs of SNPs of varying lengths. Our goals are to expand current methodology to aid in the analysis of apparent homozygous runs of SNPs with applications to data cleaning in genome-wide association studies. We analyzed apparent homozygous runs in multiple, high-density genome-wide association studies. We defined apparent homozygous runs in a manner that partially accounted for the possibility of genotyping errors within the run. At least four possible explanations for apparent homozygous runs need to be considered: 1) genuine homozygosity, 2) inherited hemizygous deletion, 3) de novo hemizygous deletion, and 4) for cell line data, loss of heterozygosity in the cell line. Towards this end, we used the genotyping intensity data to attempt to distinguish the first possibility from the other three. Using family data, when those were available, we attempted to distinguish the second possibility from the last two. Our preliminary analyses have identified long apparent homozygous runs in some HapMap study subjects. In some subjects, the longest apparent homozygous run accounts for up to 1% of the bases in the genome. Further analyses that incorporate probe intensity data are underway. We conclude that analyses of homozygous runs of SNPs may be useful as part of the data cleaning measures in genome-wide association studies.

***COL4A1* Polymorphism Is Associated With Pulse Wave Velocity By Genome Wide Association Scan.** *K. V. Tarasov[1,2], S. Sanna[3], A. Scuteri[4], J. B. Strait[1,2], M. Orrù[3], A. Parsa[5], P.-I. Lin[5], A. Maschio[3], W. Post[6], A. Cao[3], R. Nagaraja[2], B. Mitchell[5], G. Abecasis[7], A. R. Shuldiner[5], M. Uda[3], E. G. Lakatta[1], S. S. Najjar[1], D. Schlessinger[2]* 1) Laboratory of Cardiovascular Science, National Institute on Aging, Baltimore, USA; 2) Laboratory of Genetics, National Institute on Aging, Baltimore, USA; 3) Istituto di Neurogenetica e Neurofarmacologia (INN), Cagliari, Italy; 4) Unitá Operativa Geriatria, (INRCA), Rome, Italy; 5) Department of Medicine, University of Maryland School of Medicine, Baltimore, USA; 6) Department of Medicine, Johns Hopkins School of Medicine, Baltimore USA; 7) Center for Statistical Genetics, University of Michigan, Ann Arbor, USA.

Pulse wave velocity (PWV), a non-invasive index of central arterial stiffness, is a potent predictor of cardiovascular mortality and morbidity. Heritability and linkage studies have pointed toward a genetic component affecting PWV. We conducted a genome-wide association study (GWAS) to identify SNPs associated with PWV. The study cohort included participants from the SardiNIA study for whom PWV measures were available. Genotyping was performed in 4,221 individuals, using either the Affymetrix 500K or the Affymetrix 10K mapping array sets (with imputation of the missing genotypes). The associations with PWV were evaluated using an additive genetic model that included age, age^2 and sex as covariates. The findings were tested for replication in an independent internal Sardinian cohort of 1,828 individuals, using a custom-chip designed to include the top 43 non-redundant SNPs associated with PWV. Of the loci that were tested for association with PWV, a SNP in the *COL4A1* gene on chromosome 13 and a SNP in the *MAGI1* gene on chromosome 3 were successfully replicated ($p=2.0x10^{-7}$ and $p=4.5x10^{-6}$ respectively for the combined analyses). The association between SNP in *COL4A1* and PWV was also successfully replicated (p=0.02) in an independent population, the Old Order Amish, leading to an overall p-value of $1.43x10^{-8}$. Collagen type IV is the major structural component of basement membranes, suggesting that previously unrecognized cell-matrix interactions may exert an important role in regulating arterial stiffness.

Role of PALB2 in familial melanoma and pancreatic cancer. *N. Sabbaghian[1,2], N. Hamel[3], R. Kyle[1,2], H. Rothenmund[4], A. Hao[5], D. Hogg[5], S. Gallinger[4], W. Foulkes[1,2,3], M. Tischkowitz[1,2]* 1) Program in Cancer Genetics, Departments of Oncology and Human Genetics, McGill University, Montreal, Quebec, Canada; 2) Segal Cancer Centre, Sir M.B.Davis Jewish General Hospital, Montreal, Quebec, Canada; 3) The Research Institute, McGill University Health Centre, Montreal, Quebec, Canada; 4) Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Ontario, Canada; 5) Department of Medical Biophysics, Institute of Medical Sciences, University of Toronto, Toronto, Ontario, Canada.

PALB2 is a recently identified breast cancer susceptibility gene whose protein is closely associated with BRCA2 and is essential for BRCA2 anchorage to nuclear structures. This functional relationship made PALB2 a candidate gene for susceptibility to BRCA2-related cancers such as melanoma and pancreas cancer. The purpose of this study was to screen for the presence of germline mutations in PALB2 in familial melanoma families and in families with pancreatic cancer, thus assessing its role in these cancers. Methods. We sequenced PALB2s exons and introns boundaries in probands from 53 families with familial melanoma where p16 mutations were absent and 80 individuals with pancreatic cancer and at least one first or second degree relative with pancreas cancer (n=48), breast (n=23) or prostate cancer (n=9) with no known mutations in BRCA2. We screened a further nine individuals with pancreas and other primary cancers. Results. No significant variants were found in the melanoma study and one unreported silent variant,V398V, was identified in one case from the pancreatic cancer cohort. No truncating mutations were identified in either group. Conclusions. These results indicate that deleterious PALB2 mutations are unlikely to play a significant role in familial melanoma or pancreatic cancer.

Interrogating genomic region containing major effect risk gene for renal disease for signatures of recent selection in human populations. *T. K. Oleksyk[1,2], G. W. Nelson[1,2], M. Jamba[1,2], C. A. Winkler[1,2], M. W. Smith[1,2]* 1) Laboratory of Genomic Diversity, NCI, Frederick, MD; 2) Basic Research Program, SAIC, Frederick, MD.

MYH9 encodes nonmuscle myosin IIA, and is widely expressed in most tissues. Mutations in MYH9 are associated with May-Heggalin, Sebastian, Fechtner, and Epstein syndromes that all involve autosomal dominant macrothrombocytopenia. In addition, recently MYH9 has been associated with focal segmental glomerulosclerosis and renal disease. Several recent whole-genome scans indicated that MYH9 has been the subject of natural selection during different periods of human evolution, starting from time of human-primate divergence, to the recent local adaptations. We investigated the role of population history and natural selection in shaping genetic diversity in the MYH9 gene region in a group of worldwide and ethnologically well-defined human populations. We first examined evolutionary history early in the human lineage by comparing sequence of the gene in humans to the sequences of its primate relatives (chimpanzee, orangutan, and rhesus macaque). Then, we determined sequence diversity patterns, linkage disequilibrium and haplotype divergence of the MYH9 gene in the Human Diversity Panel. Finally, we concentrated on the genomic neigborhood to evaluate (1) the extent of haplotype homozygosity, (2) patterns of sequence polymorphism and (3) population divergence in the gene as well as its neighboring region. Understanding haplotype structure, evolutionary history and the role of natural selection in shaping disease genes like MYH9 is a crucial next step in biomedical reseach.

Expanding the phenotype of 22q11.2 microduplication. *E. Prijoles[1], M. Sutcliffe[1,2], J. Ranells[1]* 1) Department of Pediatrics, Division of Medical Genetics, University of South Florida, Tampa, FL; 2) Department of Pathology, All Children's Hospital, Saint Petersburg, FL.

Clinical reports of 22q11.2 microduplication (OMIM #608363) are limited compared to the reciprocal 22q11.2 microdeletion syndrome. Chromosome 22q11.2 microduplication shows phenotypic overlap with DiGeorge/velocardiofacial syndrome, but the phenotypic spectrum is proving to be much broader. We present a patient with additional features. In infancy, large fontanelles, right frontal upsweep, prominent eyes with shallow orbits, preauricular pits, prominent maxilla, thin vermilion border with ill-defined philtrum, undescended testes, hypoplasia of the fourth and fifth toes,hoarse hypernasal cry, mild hearing loss, and neonatal hepatitis were noted. Optic atrophy, Duane anomaly type II, and bilateral lacrimal punctal atresia were detected on ophthalmology exam. Brain MRI revealed normal optic nerves and chiasm and partial interrupted pituitary stalk. Growth hormone stimulation studies showed low peak growth hormone response. At age 16 years, the patient had severe cognitive impairment, poor coordination, attention deficit hyperactivity disorder, short stature, hypertelorism, upslanted palpebral fissures, small low-set ears, bifid uvula, broad neck with low posterior hairline, squared fingertips, short fourth toes with distal hypoplasia, and pes planus. Chromosomal microarray analysis (CytoChip) showed a gain in copy number at chromosome 22q11.21, detected with 7 clones encompassing at least 0.66 Mb. All clones were within the DiGeorge syndrome locus. Parents were not available for testing, although family history was significant for learning problems in both parents and emotional difficulties, bilateral atresia of lacrimal puncta, cleft lip and short fourth toes in the mother. Lacrimal punctal atresia, Duane anomaly, and pituitary stalk abnormalities with growth hormone deficiency have not been previously reported in 22q11.2 microduplication. These characteristics further expand the highly variable phenotype seen in this condition. Additional research may ultimately provide a molecular basis for the broad range of clinical features observed in 22q11.2 microduplication.

Detection of a 3q29 microdeletion syndrome case diagnosed by CGH-array. *AL. Silva, J. L'Heureux, S. Daack-Hirsch, JC. Murray* Dept Pediatrics, Univ Iowa, Iowa City, IA.

The identification of microdeletions syndromes started with the recognition of syndromic phenotypes associated with mental retardation and have been evolving with the use of more advanced molecular technologies especially the use of microarrays. These comprehensive arrays make it possible to identify not only new syndromes, but also characterize new features for already described conditions. Here we report a case of 3q29 microdeletion syndrome identified with the use of Affymetrix Genome-Wide Human SNP Array 6.0 that includes more than 906,600 single nucleotide polymorphisms (SNPs) and more than 946,000 probes for the detection of copy number variation. The patient phenotype includes broad nasal root, cleft lip and palate, complete syndactyly of third and fourth toes on the right foot, cup shaped ears, thickened helices and developmental delay. The analysis shows a 1.5 Mb microdeletion at the 3q29 region; the same region reported previously by Willat et al. (2005). The deletion encompasses 22 genes including PAK1 and DLG1 that are the autosomal homologues of two known X-linked mental retardation genes, PAK3 and DLG3. The syndrome phenotype is variable despite the almost identical deletion size in all cases. Our study confirms that microarray analysis is a very important tool for screening and detection of submicroscopic chromosomal abnormalities that are undetectable by current cytogenetic and/or molecular methods. We report only the 7th case of the 3q29 microdeletion syndrome and add more description to its phenotype.

High Carrier Frequency of Founder Mutation Causing Severe/Lethal Recessive Type VIII Osteogenesis Imperfecta in West Africans and African-Americans. *W. A. Cabral[1], A. M. Barnes[1], C. N. Rotimi[2], L. Brody[3], J. Bailey-Wilson[3], S. R. Panny[4], D. Chitayat[5], F. D. Porter[6], J. C. Marini[1]* 1) Bone and Extracellular Matrix Branch, NICHD, NIH, Bethesda, MD; 2) ICGHD, NHGRI, NIH, Bethesda, MD; 3) NHGRI, NIH, Bethesda, MD; 4) Maryland DHMH, Baltimore, MD; 5) Dept OB/Gyn, Mt Sinai Hosp, Toronto, Ontario; 6) HDB, NICHD, NIH, Bethesda, MD.

Type VIII OI (OMIM #610915) is a lethal or severe recessive form of OI caused by mutations in *LEPRE1*, the gene encoding prolyl 3-hydroxylase 1. We identified a recurring mutation, IVS5+1G>T, in 6 probands born to carrier parents of West African or African-American descent, suggesting an African founder mutation which had been transported to the Americas. We investigated the carrier frequency for this mutation in African-American and contemporary West African populations and the molecular anthropology of the mutation. We screened gDNA using PCR and RE digestion, or a custom SNP assay, followed by PCR confirmation of positive samples. The recurring mutation was identified in 5/995 Washington DC, 5/1429 Pennsylvania and 2/631 Maryland samples. Thus, Mid-Atlantic African-Americans have a carrier rate of 1/200-300 and a predicted incidence of homozygosity for this mutation of 1/160,000-380,000 births. Fifteen of 1097 unrelated individuals (1.37%) from Nigeria and Ghana were heterozygous for *LEPRE1* IVS5+1G>T, all but one from Kwa-speaking tribes. The high (>1%) carrier frequency for this founder mutation among West Africans predicts an incidence of recessive OI in West Africa of 1/21,000 births, which is equal to the incidence of *de novo* dominant OI, and an order of magnitude greater than the 5-7% proportion of all recessive OI in North America. To estimate mutation age, we used microsatellites and short tandem repeats covering 4.2 MB surrounding *LEPRE1* on chromosome 1p to characterize the conserved haplotype. Haplotype analysis of the founder mutation pedigrees has revealed a conserved region of <450Kb, consistent with a single mutation that arose over 300 years ago and was transported by the Atlantic slave trade. We estimate that 350 carriers were transported to the American colonies, preventing the occurrence of a secondary founder effect.

Non-recipient DNA in squamous cell carcinomas of transplant patients. *A. Toland*[1], *A. Dworkin*[1,2] 1) MVIMG/Human Cancer Genetics, Ohio State University, Columbus, OH; 2) IBGP Program.

Background: Organ transplant recipients (OTRs) have a 65-80 fold increased risk of cutaneous squamous cell carcinoma (SCC). There is a great deal of variability in SCC development in OTRs, suggesting genetic contributions to risk. For example, years post transplant some OTRs develop up to 20 tumors while others do not develop any, despite similarities between other risk factors. In previous studies, we showed that some tumor susceptibility loci demonstrate allelic selection during tumor development and that allelic imbalance studies can be used to identify these loci. To identify loci showing preferential imbalance in SCCs from OTR, we genotyped paired normal and tumor DNA from individuals with five or more SCCs using microsatellite markers from loci exhibiting frequent losses or gains in SCCs. In 11 of 44 individuals, we observed extra alleles in some tumors when comparing tumor genotypes to normal DNA genotypes from the recipient. We never observed extra alleles in any SCCs from non-OTRs. These data made us consider the possibility that the OTR SCCs contained DNA of donor origin. **Methods and Results:** To examine this possibility, we tested matched normal and tumor DNA from 5 females showing extra genotyping bands for the presence of Y-chromosome specific SRY markers. Tumors from 3 of the 5 females were PCR positive for SRY. All of the female controls and some other SCCs from these patients were negative for SRY. Next, we genotyped matched normal and tumor DNA from 6 males and the 2 females negative for SRY for X chromosome markers. Tumors from 2 of the 6 males and 1 of the 2 females showed 2 or more additional bands for X-specific markers suggesting they may have had an organ donation from a female donor. Quantitation of the levels of non-OTR versus OTR DNA by comparison of peak height of alleles suggests that the non-recipient DNA comprises 20-50% of the total DNA. **Conclusions:** SCCs from OTRs contain DNA of non-recipient origin. Ongoing studies are to confirm that the DNA is of donor origin and to identify the cell type of origin. This work has implications for the role of stem cells or immune cells from organ donors in cutaneous SCC development.

Nine years of experiences of Integrated Prenatal Screening at North York General Hospital, Toronto, Ontario, Canada. *A. M. Summers[1, 2], T. Huang[1], K. Boucher[1], S. Rashid[1]* 1) Genetics Program, North York General Hosp, Toronto, ON, Canada; 2) Department of Pediatrics, Faculty of Medicine, University of Toronto, Toronto, ON, Canada.

Integrated Prenatal Screening (IPS) was first started in Ontario at North York General Hospital (NYGH) in December 1999. The first step of IPS involves an ultrasound for nuchal translucency and a blood sample for maternal serum pregnancy-associated plasma protein A at 11-14 weeks gestation. The second step is a blood sample at 15 - 20 weeks for maternal serum AFP, uE3 and total hCG. The result is based on the integration of the two parts. Prior to the introduction of IPS, a screening protocol, patient brochure, counselling guideline and requisition were developed. Education sessions were organized to educate health care providers throughout the NYGH catchment area. Between December 1999 and June 2007, 81,779 women were enrolled in our IPS program. 68,026 women completed 5-marker IPS. Others had 6-marker IPS and serum IPS. Six percent of the women did not complete the second part of IPS. Of women who completed IPS, the detection rate for Down syndrome was 84.9 % and the false positive rate was 2.7 %, which is consistent with the literature. For every 14 women who were screen positive, one case of Down syndrome was diagnosed. IPS represented 62% of our current screening volume, followed by Quad test (22%) and First Trimester Screening (9%). IPS is now offered at all screening centres in Ontario as part of Enhanced Prenatal Screening funded by the Ontario Ministry of Health and Long-Term Care. As of April 2008, 214,819 women have IPS and over 700 sonographers have been enrolled into the Ontario program. Since the introduction of enhanced screening, the overall screening uptake rate in Ontario has increased from 47% in 2002 to 62% in 2007. Our experience shows that IPS is feasible and is acceptable to pregnant women and health care providers at NYGH and in Ontario.

Double mutant mouse for Dystrophin and Large proteins: a new model for neuromuscular diseases. *M. Vainzof[1], P. C. M. Martins[1], V. L. Ferreira[1], D. Ayub-Guerrieri[1], P. C. Onofre[1], C. M. C. Mori[2], L. U. Yamamoto[1]* 1) Hum Genome Res Ctr, IB-Univ Sao Paulo, Sao Paulo, Brazil; 2) Depto Patologia, Veterinary Inst. Univ Sao Paulo, SP, Brazil.

The muscular dystrophies (MD) represent some of the most common human genetic disorders yet few treatment options are available. Animal models are therefore a major tool for testing therapies. Several natural mice models for MD are recognized. The dystrophin-deficient *mdx* mouse is the most widely used model for Duchenne muscular dystrophy. However, because of its very mild phenotype, it is less informative in clinical trials. The myo-dystrophy mouse, the murine model of congenital CMD1D, harbors a mutation in the glycosyltransferase *large* gene, which leads to altered glycosylation of -DG, and a severe phenotype. Aiming the use of these animal models in therapeutic trials, we are defining the clinical natural history and standardizing tests for the analysis of muscle strength and motor ability. Additionally, to help to elucidate the role of the proteins dystrophin and large in the organization of the dystrophin-glycoprotein-complex in muscle sarcolemma, we generated through mendelian cross breads, double mutants mice for dystrophin/large proteins. Up to the present date, 78 animals from 9 litters were born from 6 mdx/double heterozygous couples: 15 (19%) normal, 43 (55%) heterozygous and 20 (26%) double affected puppies. Therefore, this double mutant is compatible with life, and no intrauterine dead apparently occurred. About 75% of the affected puppies died within the first 24 hours, suggesting a strong selection in the peri-natal period. Five animals remained alive and are showing a clinical course worst that the original Large model of the same age. Histological and histochemical and immunohistochemical analysis of the muscle are elucidating the involved histopathological alterations. These new animal models carrying two simultaneous mutations can be used as important tools for genetic, clinical studies, providing important clues to the understanding of the pathogenesis of these disorders. FAPESP-CEPID, CNPq, ABDIM-BR.

MYP3 Locus for High-grade Myopia: Association Analyses and Mutation Screening. *R. Metlapally[1,2], K. N. Tran-Viet[2], T. R. White[1,2], A. Bulusu[2], B. Zhao[2], J. Zhou[2], G. R. Czaja[2], Y.-J. Li[2], T. L. Young[1,2]* 1) Duke Univ Eye Center, Durham, NC; 2) Duke Ctr Human Gen, Durham, NC.

Purpose: The first MYP3 locus (12q21-23) for autosomal dominant high-grade myopia was mapped in a large family of German/Italian origin, and was replicated in two subsequent small family studies. Our recent whole genome linkage scan using single nucleotide polymorphisms (SNPs) in a 5-site international Caucasian dataset of 249 multiplex high-grade myopia families also confirmed this locus. To identify the implicated MYP3 gene, we employed a two-pronged approach that included SNP-based association analysis to refine the interval, and sequence variant screening. **Methods:** 239 SNPs (average spacing 33.8 kilobases) for an 8 megabase (Mb) region were selected for an Illumina Goldengate genotyping assay based on coding function, biological relevance, ranking score, validation, and frequency information. A total of 657 subjects from 146 multigenerational high-grade myopia families participated in the study. Genotype data were checked and corrected for Mendelian inconsistencies. Markers were checked for Hardy-Weinberg equilibrium and linkage disequilibrium between markers was calculated. Family-based pedigree disequilibrium test (PDT), GenoPDT, and association in the presence of linkage test were used for testing SNP association with high myopia. Eight genes were sequenced in 12 affected and unaffected subjects from 3 families that showed strongest linkage to MYP3 in our linkage study. **Results:** Family-based association analyses revealed significant association of several SNPs from the 91-94 Mb interval on chromosome 12 ($p<0.01$). Based on significant association values and expression in ocular tissues, we prioritized 8 candidate genes for mutation screening: ATP2B1, EEA1, MRPL42, PLXNC1, CCDC41, TMCC3, FGD6, and C12orf63. Sequence analysis did not reveal segregated variants with affection status. **Conclusions:** The SNP-based analyses revealed potential involvement of a 3 Mb region in the MYP3 locus. Mutation analysis of 8 candidate genes in this region did not identify sequence variants associated with the MYP3 high myopia phenotype. Further screening of candidate genes is underway.

Common Genetic Variants in Candidate Genes and Risk of Familial Lymphoma. *X. Liang, D. Ng, M. L. McMaster, O. Landgren, N. Caporaso, L. R. Goldin* Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD.

A role for genetic factors in chronic lymphocytic leukemia (CLL) is unequivocal based on evidence from multiply affected families, case series and twin, case-control and population-based registry studies. Similar findings have been reported for Hodgkin lymphoma (HL), non-Hodgkin lymphoma (NHL) and WaldenstrÖm macroglobulinemia (WM). Population-based studies have found CLL, HL, NHL, and WM to co-occur in families, suggesting that shared genetic pathways may contribute to risk for multiple lymphoproliferative malignancies. Using a custom-designed Illumina panel containing 1536 SNPs, we investigated the effect of common genetic variants in 152 candidate genes from several functional groups including apoptosis, DNA repair, immune response, and oxidative stress among 44, 50, 28, and 71 unrelated familial CLL, HL, NHL, and WM patients from high-risk families, respectively, and a control group consisting of 107 spouses from the same families. Several findings were notable. SNPs in BCL2 showed an association with all four phenotypes. SNP rs9989529/BCL2 was significant in both NHL and WM with ORs of 2.28 (95% CI: 1.19-4.37) in NHL and 1.66 (95% CI: 1.08-2.56) in WM. SNP rs4987827/BCL2 was significant in both NHL and HL. Polymorphisms in IL10 (including a variant in the promoter region previously reported to be associated with NHL), TRAIL and TRAILR1 were found associated with CLL and WM. Consistent with prior data, IL6 polymorphisms were associated with WM and HL. To our knowledge, this is the first large-scale dense-SNP candidate gene study in familial lymphomas. Our results highlight the importance of both extrinsic and intrinsic apoptosis pathways in lymphoma etiology. Future investigations are needed to replicate our findings and to define functional roles of genetic variants associated with the risk of developing lymphoproliferative malignancies.

Correlation of CpG Methylation with Gene Expression in Patients with Coronary Artery Disease. *H. Tao[1], P. L. Beineke[1], S. E. Daniels[1], W. E. Kraus[2], E. R. Hauser[2], S. Rosenberg[1], J. A. Wingrove[1]* 1) CardioDx, Palo Alto, CA; 2) Duke University, Durham, NC.

We have previously shown that gene expression in circulating blood cells significantly correlates with severity of coronary artery disease (CAD). Epigenetic modifications, including CpG methylation, are known to influence gene expression, prompting us to investigate whether CpG methylation might influence gene expression in relation to CAD. To study this possibility, we measured CpG methylation in two sets of samples, choosing five genes whose expression levels have previously been shown to correlate with CAD severity: TLR5, S100A9, CAPG, TNFSRF1b, and SERPINB6. A total of 17 amplicons were used to interrogate upstream promoter regions spaced in ~ 1 Kb increments beginning at the transcriptional start site of each gene. Amplicons averaged 180 bp in length and contained an average of 4 CpGs. Genomic DNA was purified in 2 sets from 231 patients obtained from the CATHGEN registry (a biorepository paired with clinical and angiographic data in the Duke Databank for Cardiovascular Disease) and was subjected to bisulfite modification. Quantitative High Resolution Melting (HRM) was then used to estimate the degree of methylation in each of the amplicons across the first set of 143 samples. After adjusting for age, gender, ethnicity and diabetes status in a multiple linear regression model, methylation in 3 amplicons was significantly associated with expression levels as determined by RT-PCR ($p < 0.05$). A region in the TLR5 promoter showed the strongest association ($p < 0.001$), leading us to examine 7 additional loci in the TLR5 gene, of which 1 locus showed significant correlation ($p < 0.01$). When re-assessed in the second set of 88 patient samples, both loci identified in the first set were again significantly associated with expression of TLR5 ($p < 0.01$). In conclusion, we have identified two regions in the TLR5 promoter that show significant correlation between gene expression and CpG methylation levels, across 2 sets of samples. This result suggests that epigenetic modifications may play a role in regulating gene expression in CAD.

Genome-wide targets of aberrant methylation in serous epithelial ovarian cancer cells. *D. Biscocho, J. J. Connelly, Z. Huang, S. K. Murphy, S. G. Gregory* Duke University, Durham, NC.

Ovarian cancer is an insidious disease that has usually metastasized prior to diagnosis. Abnormal DNA methylation is frequently found at specific loci in ovarian cancers and may provide opportunities to improve early disease detection and treatment. Unlike genetic mutations, aberrant DNA methylation is potentially reversible via epigenetic-based therapies. We have utilized DNA tiling path arrays to determine the genome-wide distribution of methylation in cell lines derived from spontaneously transformed normal ovarian surface epithelium (NOSE-06) and malignant ovarian cancer (SKOV4). 2414 regions of the genome were identified as differentially methylated (p0.01) in SKOV4 versus NOSE-06. Several regions contained genes known to be aberrantly methylated in ovarian cancer cells lines (*GSTP1*, *FLNC*, *CDH1*, *MAGEA3*, and *PYCARD*). Interestingly, loci previously identified as methylated in ovarian cancer cell lines were methylated in NOSE-06. It is not known how spontaneous immortalization and passaging in cell culture have influenced the methylation profiles of NOSE-06, but suggests the importance of defining normal methylation profiles of relevant tissues in order to serve as a basis for comparison to ovarian malignancy. We have validated the methylation state of several novel genes using methylation specific PCR (*CDH2*, *CDH4*, *PLAUR*, *CALCB*, *PLEK2*, *FN1*, *NR2E1*). Correlative gene expression analysis identified two genes that exhibit a decrease in gene expression and an increase in promoter methylation in two separate ovarian cancer cell lines. Interestingly, these genes, fibronectin 1 (*FN1*) and alpha-actinin 1 (*ACTN1*), both play a role in cell adhesion and migration. Pathway analysis of the genes contained within differentially methylated regions indicated enrichment of pathways involved in tight junction (p=0.01) and adherens junction (p=0.05) formation. Disturbance of the integrity of intercellular junctions promotes invasiveness and mobility of cancer cells. Taken together this information indicates the role DNA methylation may play in the integrity of intercellular junctions and implicates a reversible cellular process in the oncogenic transformation of a cell.

Somatic reversion? A case of mosaic dup(17)(p11.2p11.2) in a mother and child. *R. Yusupov[1,4], A. Roberts[1,2,4], R. V. Lacro[2,4], M. Sandstrom[3], A. H. Ligon[3,4]* 1) Division of Genetics, Children's Hospital Boston, MA; 2) Department of Cardiology, Children's Hospital, Boston, MA; 3) Department of Pathology, Brigham and Women's Hospital Boston, MA; 4) Harvard Medical School Boston, MA.

Low copy repeat (LCR) sequences in 17p11.2 predispose this region to genomic deletions and duplications. Duplication of 17p11.2, also known as Potocki-Lupski syndrome (PTLS), is a recently recognized contiguous gene syndrome featuring cognitive and language deficits, developmental delay, autistic behavior, structural cardiovascular anomalies, hypotonia, failure to thrive, apnea and dysmorphism. We present a mother and child, both mosaic for dup(17)(p11.2p11.2), who share dysmorphic features. At birth, the infant was noted to have microcephaly, prominent occiput, short and down-slanting palpebral fissures, deeply set eyes, epicanthal folds, micro and retrognathia, a long, bulbous nasal tip, hypotonia and a grade II/VI harsh systolic murmur. Echocardiogram showed hypoplastic left heart syndrome with mitral and aortic atresia, hypoplastic ascending aorta, and a large patent ductus arteriosus, confirming the prenatal diagnosis. Considerable oral motor and pharyngeal phase swallowing dysfunction was present. Mosaicism for the dup(17)(p11.2p11.2) was suspected initially by GTG-banded analysis of metaphase chromosomes from peripheral blood specimens of both individuals. This suspicion was subsequently confirmed by interphase fluorescence in situ hybridization (FISH) using a probe within the Smith-Magenis critical region (17p11.2)and a control probe mapping to 17p12. In both the child and mother, the dup(17) was identified in ~60-70% of nuclei. Confirmation of this cytogenetic duplication was supported by array comparative genomic hybridization (aCGH). The fact that this aberration was visible cytogenetically suggests that the duplicated region is greater than 5Mb. Here we provide a thorough description of the phenotypes of each affected individual, and propose a mechanism by which mosaicism for the duplication could exist in individuals from two successive generations.

Sequencing reveals low variability in human microRNA genes. *C. H. Lee[1], L. Pennachio[2], A. Pertsemlidis[1]* 1) The University of Texas Southwestern Medical Center, Dallas, TX; 2) DOE Joint Genome Institute, Walnut Creek, CA.

MicroRNAs (miRNAs) are short (~21 to 23 nucleotides) RNAs that regulate gene expression by binding to the 3' untranslated region (3'-UTR) of their target mRNAs, resulting in either modulation of translational efficiency or in degradation of the mRNAs. miRNAs have been shown to play important roles in embryonic development as well as human diseases, including cancer. We sequenced nine loci containing miRNAs predicted to target genes related to lipid and sterol homeostasis in 1,917 human subjects. These subjects were drawn from the Dallas Heart Study (DHS), a single-site, multi-ethnic, population-based probability sample. We sequenced a total of 7.2 kb of genomic DNA, covering 17 mature miRNAs and 13 miRNA passenger strands. We identified 115 single nucleotide polymorphisms (SNPs) and 8 insertion/deletion polymorphisms (indels); the majority (89 of 123) of these polymorphisms were extremely rare, with minor allele frequencies of less than 0.01 across all ethnicities. The density of SNPs within mature microRNAs and passenger strands (~3.5 SNPs per kb) is much lower than the density within other regions of the miRNA precursors and the flanking regions (~7 to 12 SNPs per kb, depending on ethnicity), as is , the average per nucleotide heterozygosity (on the order of 10^{-5} for mature miRNAs and passenger strands and 10^{-4} for other precursor and flanking regions). Sequencing of approximately 100 other miRNA loci across a smaller sample of 32 African American subjects from the DHS confirmed the rarity of SNPs in mature miRNAs and their passenger strands. Several of the SNPs typed in this study showed associations with measured levels of plasma lipids, suggesting a role for miRNAs in lipid and sterol homeostasis; these associations match observations made in other published genome-wide association studies (GWAS). Together, these data provide evidence of selective pressure imposing sequence constraints on miRNAs and their precursors, as variation may affect overall miRNA expression, thereby altering the regulation of specific target genes.

Identification of a novel mutation in *LAMIN A/C* in a family with atrial fibrillation, progressive conduction system disease and dilated cardiomyopathy. *H. Pan[1], A. A. Richards[1], J. Chen[2], J. A. Joglar[2], H. L. Yin[3], V. Garg[1, 4], UT Southwestern, Dallas, TX* 1) Department of Pediatrics; 2) Department of Internal Medicine; 3) Department of Physiology; 4) Department of Molecular Biology.

Atrial fibrillation (AF) is the most common type of arrhythmia in adults, and it infrequently occurs in association with conduction abnormalities and dilated cardiomyopathy (DCM). We identified a family spanning 3 generations with autosomal dominant cardiac disease that had 15 affected members. Clinical histories were obtained by review of available medical records. Specifically, 12 subjects had AF, 12 had atrioventricular block (AVB), 10 had DCM and 8 experienced sudden cardiac death (SCD). In the majority of cases, the first symptom of disease in affected family members was a cardiac conduction abnormality, either AF or AVB, occurring between 30 and 55 years of age. DCM was always diagnosed after the initial presentation and in 5 cases with a delay of at least 8 years. Similarly, SCD occurred 5 to 30 years after the initial presentation. The subjects were studied to determine the genetic etiology of their cardiac disease. Genomic DNA was collected from available family members after obtaining informed consent. Genetic studies excluded linkage to all previously described AF loci except for the lamin A/C (*LMNA*) locus. Direct sequencing of the *LMNA* gene in affected members identified a heterozygous single base pair insertion after nucleotide 348 (c348_349insG). Consistent with the mutation being disease-causing, it segregated with affected members and resulted in a frameshift that predicted premature truncation of the protein. The observed K117fs mutation in *LMNA* is novel and highly penetrant. Mutations in *LMNA* have been described in a group of heterogeneous diseases, termed laminopathies, which affect multiple organ systems. The identification of a novel mutation in *LMNA* in this pedigree with isolated cardiac disease highlights the importance of screening for mutations in *LMNA* in patients with isolated AF, especially those with a positive family history for DCM or SCD. Additional studies of this *LMNA* mutation may suggest a mechanism for the development of AF, AVB and DCM in affected individuals.

Waved with open eyes (*woe*) phenotype caused by a mutation in *Adam17*. *E. L. Hassemer[1], R. R. Dubielzig[2], C. Zeiss[3], B. Chang[4], D. J. Sidjanin[1]* 1) The Department of Cell Biology, Neurobiology and Antomy, Medical College of Wisconsin, Milwaukee, WI 53226; 2) School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI 53706; 3) School of Medicine, Yale University, New Haven, CT 06520; 4) The Jackson Laboratories, Bar Harbor, ME 04609.

Waved with open eyes (*woe*) is an autosomal recessive mouse mutant that arose spontaneously on the C57BL/6J background. Phenotypically, the woe mice have a wavy coat, microphthalmia/anophthalmia and corneal opacities. In order to map the *woe* locus, homozygote woe mice were backcrossed to C3H mice to generate 138 F2 progeny. A genome wide scan mapped the woe locus to the proximal arm of mouse chromosome 12. Evaluation of the *woe* critical region identified *Adam17* as a candidate gene. The role of Adam17 is in shedding the soluble forms of TNF-, TGF- and other proteins from their membrane-bound precursors. The $Adam17^{-/-}$ mice are phenotypically similar to the *woe* mice; however, $Adam17^{-/-}$ mice die shortly after birth. The Adam17 sequence analysis in *woe* mice revealed a C794T substitution that leads to a Thr265Met change in an evolutionary conserved residue. To prove that the $Adam17^{Thr265Met}$ substitution is responsible for the woe phenotype, a complementation breeding was set up with an $Adam17^{+/-}$ mouse. The Adam17 null allele rescued the lethality seen in the $Adam17^{-/-}$ mouse, but did not rescue the eye and fur phenotype proving the mutation. Western blot analysis did not detect different levels of Adam17 in wild-type and woe tissues. *In-vitro* expression and subcellular localization of $Adam17^{Thr265Met}$ in COS-7 cells showed no difference in the subcellular localization between $Adam17^{Thr265}$ and $Adam17^{Thr265Met}$; however, evaluation of the free TNF- in sera of woe and wild-type mice via ELISA showed a slight decrease in $Adam17^{Thr265Met}$ shedding activity indicating that the mutation might be affecting other Adam17 specific substrates (e.g. TGF-). Our data suggest that *woe* is a hypomorphic mutation in Adam17 where the Thr265Met substitution results in lowering the metalloprotease enzymatic function. Evaluation of the role of Adam17 in ocular development is currently in progress.

Psoriasis Association with ZNF313-SPATA2 Region on Chromosome 20q13 Replicated. *R. P. Nair[1], P. E. Stuart[1], T. Tejasvi[1], H. W. Lim[2], E. Christophers[3], J. J. Voorhees[1], J. T. Elder[1]* 1) Department of Dermatology, University of Michigan, Ann Arbor, MI; 2) Henry Ford Hospital, Detroit, MI; 3) University of Kiel, Kiel, Germany.

Psoriasis is a common inflammatory skin disease with genetic, immunological and environmental inputs. Several linkage studies and recent genome-wide association studies have mapped the major genetic determinant of psoriasis susceptibility to the vicinity of HLA-C. Replicated association studies have confirmed the involvement of IL12B and IL23R in psoriasis, providing an additional genetic basis for inflammatory and immune processes in psoriasis. Recently Capon et al. (Hum Mol Genet 13:1938, 2008) reported a novel association of psoriasis to rs495337, a synonymous coding SNP located in SPATA2, which is in absolute linkage disequilibrium with ZNF313 on chromosome 20q13. Unlike SPATA2, ZNF313 is abundantly expressed in skin, T cells and dendritic cells, leading the authors to suggest that ZNF313 may be the causal genetic determinant at this locus. In the present study, we attempted to replicate this association in two large psoriasis cohorts: a collection of 686 pedigrees of varying sizes (2744 individuals), and a case/control collection of 1588 cases and 1921 controls, all of Caucasian ancestry. While the pedigrees showed no association (FBAT S* = 0.017, p = 0.68), the case/control sample showed confirmatory evidence for association (OR = 1.19, p = 3.2E-04) for the same the risk allele described by Capon et al. (G). When the case control sample was enhanced using one case per family of US Caucasians (552 individuals) for a total of 2140 cases, the association results modestly improved with no change in odds ratio (OR = 1.19, p = 8.9E-05). These results confirm the association of psoriasis with the ZNF313 - SPATA2 region. Further studies of large cohorts are needed to fine map the associated haplotype(s) and identify the causal variant(s) at this locus.

The Mouse Genome Informatics (MGI) Database: A resource for genetic and phenotypic information for mutant mice as models of human disease. *H. Onda, A. Anagnostopoulos, R. Babiuk, S. Bello, D. L. Burkart, H. Dene, M. Knowlton, I. Lu, T. Meehan, B. Richards-Smith, C. L. Smith, M. Tomczuk, L. L. Washburn, J. T. Eppig, the Mouse Genome Informatics Group* Mouse Genome Informatics, The Jackson Laboratory, Bar Harbor, ME.

Mouse models of human diseases are invaluable tools for researchers for understanding disease processes and developing potential new therapies. Physiological and genetic similarities between human and mouse make the laboratory mouse a premier model for the study of human genetic disease. With the wealth of available experimental tools for the mouse, including a high-resolution genetic map, complete genome sequence, myriad inbred and mutant strains and the technologies to alter the genome, the mouse has become an essential surrogate for exploring human disease. The Mouse Genome Informatics Database (MGI, http://www.informatics.jax.org) supports biological knowledge building for the laboratory mouse by integrating and providing access to a wide range of data including phenotype and disease associations and their underlying genetic cause; gene function and genome location; gene and protein sequence; and expression data. Currently, MGI contains almost 24,000 mouse phenotypic alleles including QTL. Over 23,000 genotypes are annotated with one or more phenotypic terms; and more than 2,200 genotypic mouse models are associated with 800+ human diseases or syndromes. Phenotypic abnormalities of mutant mice and their similarities to human disease are curated using a structured vocabulary of mouse anomalies (the Mammalian Phenotype Ontology) and human disease terms from the Online Mendelian Inheritance in Man (OMIM). These standard terms provide a backbone for robust annotation, allowing comprehensive queries and supporting web-based and computational access to phenotype and disease data. The Mouse Genome Informatics Database is supported by NIH grant HG000330.

Contribution of mtDNA to the expression profiles of human cells grown in culture and in xenografts. *P. K. Dranchak[1], D. Magda[2], P. Lecane[2], J. Prescott[2], P. Thiemann[2], X. Ma[2], D. M. Toleno[1], K. D. Siegmund[3], J. G. Hacia[1]*
1) Department of Biochemistry and Molecular Biology, University of Southern California, Los Angeles, CA; 2) Pharmacyclics, Inc., Sunnyvale, CA; 3) Department of Preventive Medicine, University of Southern California, Los Angeles, CA.

Interactions between the gene products encoded by the mitochondrial and nuclear genomes play critical roles in eukaryotic cellular function. Here, we characterized metabolic properties and gene expression profiles of A549 lung cancer cells and their isogenic mitochondrial DNA (mtDNA)-depleted ^{0} counterparts grown in culture and as tumor xenografts in immune-deficient mice. Cultured A549 ^{0} cells were respiration-deficient and showed enhanced levels of transcripts relevant to metal homeostasis, initiation of the epithelial-mesenchymal transition, and glucuronidation pathways. Intriguingly, subsets of well-established HIF-regulated transcripts were both up- and down-regulated relative to the parental cell line, implying a higher complexity in HIF-mediated gene regulation than previously reported. Surprisingly, growth in culture versus xenograft has a significantly greater influence on expression profiles, including transcripts involved in mitochondria structure, aerobic and anaerobic respiration, and splicing. However, both *in vitro* and *in vivo*, mtDNA levels explained the majority of the variance observed in the expression of transcripts in glucuronidation, heat shock response, and immune surveillance related pathways. Overall, ^{0} xenografts and cell cultures provide two distinct, yet complementary, means to investigate the role of mitochondrial function in normal and pathological cellular processes under physiological and controlled growth conditions.

The CC2D2A gene is mutated in Joubert syndrome and implicated in the function of the primary cilium/basal body. *D. Doherty[1], N. Gorden[1], H. Arts[2], M. Parisi[1], S. van Beersum[2], A. Hikida[1], A. Alswaid[3], H. Ozyurek[4], E. Otto[5], C. Hutter[6], F. Farin[7], M. Dorschner[8], N. Katsanis[9], K. Owens[10], D. Raible[10], N. Knoers[2], P. Chance[1], R. Roepman[2], C. Moens[11], I. Glass[1]* 1) Dept Pediatrics, Univ Washington, Seattle, WA; 2) Dept Human Genetics, Radboud Univ Nijmegen Med Cntr, Nijmegen, Netherlands; 3) Dept Pediatrics, King Abdulaziz Med City, Riyadh, Saudi Arabia; 4) Dept Pediatrics, Ondokuz Mayis Univ, Samsun, Turkey; 5) Dept Pediatrics, Univ of Michigan, Ann Arbor, MI; 6) Dept Epidemiology, Univ of Washington, Seattle, WA; 7) Dept Environmental & Occupational Health Sciences, Univ of Washington, Seattle, WA; 8) Dept Medicine, Univ of Washington, Seattle, WA; 9) Mc-Kusick-Nathan Inst of Genetics, John Hopkins Univ School of Med, Baltimore, MD; 10) Dept Biological Structure, Univ of Washington, Seattle WA; 11) Fred Hutchinson Cancer Research Cntr, Seattle, WA.

Joubert syndrome and related disorders (JSRD) are autosomal recessive conditions characterized by hypotonia, ataxia, abnormal eye movements, intellectual disability and a distinctive mid-hindbrain malformation. Variable features include retinal dystrophy, cystic kidney disease and liver fibrosis. JSRD are included in the rapidly expanding group of disorders called ciliopathies, as 4 of the 5 gene products implicated in JSRD (NPHP1, CEP290, RPGRIP1L and TMEM67) function in the primary cilium/basal body organelle. Using homozygosity mapping in consanguineous families, we identified loss of function mutations in the CC2D2A gene in JSRD patients with and without retinal, kidney and liver disease. CC2D2A is expressed in all fetal and adult tissues tested. A nonsense mutation in the zebrafish CC2D2A ortholog (sentinel) results in pronephric cysts, a hallmark of ciliary dysfunction analogous to human cystic kidney disease. Using a yeast two-hybrid approach, we identified a physical interaction between CC2D2A and CEP290. Knockdown of CEP290 function in sentinel fish resulted in a synergistic phenotype, highlighting the functional importance of the physical interaction. This work implicates CC2D2A in cilium/basal body function and provides a model for the role of extragenic modifiers in JSRD.

PLAC1 gene structure and transcription response elements. *Y. Chen[1], M. Zalzman[1], S. L. Lee[2], J. G. Yoo[1], D. Schlessinger[1], M. S. H. Ko[1], R. Nagaraja[1]* 1) Laboratory of Genetics, National Institute on Aging, NIH, Baltimore, MD 21234; 2) College of Veterinary Medicine, Gyeongsang National University, 900 Gazwa, Jinju, Gyeongnam, Republic of Korea 660701.

PLAC1 is an X-linked placental specific gene that is conserved in both human and mouse. Large deletions encompassing the Plac1 region in mouse cause runty phenotype or stillbirths, hinting that the gene is involved in growth control; and recent studies find PLAC1 derepressed in multiple cancer tissues and linked to tumor cell proliferation. To understand the mechanism of tissue specificity and effects on growth we characterized the genomic DNA structure and defined promoter elements. Based on 5 RACE reaction we conclude that the gene contains a total of 6 exons, 3 of which are newly defined; the lie well upstream of the known exons. The 5.2 kb sequence between the newly identified exon 1 and the next gene shows strong promoter activity when fused to a luciferase reporter gene and transfected into Bewo cells, a choriocarcinoma-derived cell-line that expresses PLAC1 endogenously. This promoter fragment was fused to a fluorescent reporter, injected into fertilized oocytes and were then allowed to differentiate into blastocysts. In an outgrowth assay of these blastocysts, expression is restricted to the cells outside the outgrowth. We are currently testing the PLAC1-positive cells for trophoblast lineage markers. Sequential deletion of the 5.2 Kb DNA indicate that 500bp 5 of exon 1 contains essential elements to yield high promoter activity Several transcription factor binding sites are included in the putative promoter region, and we are further testing the inference that they are both necessary and sufficient to provide high-level specific expression in the placenta and cancer cells.

High Fidelity of Whole-Genome Amplified DNA on High-Density Single Nucleotide Polymorphism Arrays. *Y. Zhang, J. Xing, W. S. Watkins, D. J. Witherspoon, L. B. Jorde* Dept Human Genetics, Univ. of Utah, Salt Lake City, UT.

Current microarray technology allows researchers to genotype a large number of SNPs with relatively small amounts of DNA. Nevertheless, researchers and clinicians still frequently face the problem of acquiring enough high-quality DNA for analysis. Whole-genome amplification (WGA) methods offer a solution for this problem, and earlier studies showed that WGA samples perform reasonably well in small-scale genetic analyses (e.g. Affymetrix 10K array). To determine the performance of WGA products on a large-scale genotyping array, we compared the Affymetrix 250K array genotyping results of genomic DNA and its WGA products from four individuals. Our results indicate that WGA product performs well on the 250K array compared to genomic DNA, especially when using the BRLMM calling algorithm. WGA samples have high call rates (97.5% on average, compared to 99.4% for genomic DNA) and excellent concordance rates with their corresponding genomic DNA samples (98.7% on average). In addition, no apparent systematic genomic amplification bias can be detected. This study demonstrates that, although there is a slight decrease in the total call rates, WGA methods provide a reliable approach for increasing the amount of DNA samples for a commonly used SNP genotyping array.

Chromosome 22q13.3 deletion syndrome with a *de novo* interstitial 22q13.3 cryptic deletion disrupting *SHANK3*.

A. Delahaye[1,2], A. Toutain[3], A. Aboura[2], C. Dupont[1,2], A. C. Tabet[2], B. Benzacken[1,2], J. Elion[2], A. Verloes[2], E. Pipiras[1], S. Drunat[2] 1) Cytogenetics Department, APHP-Jean Verdier University Hospital, Bondy, France; 2) Genetics Department, APHP-Robert Debré University Hospital and INSERM U676, Paris, France; 3) Genetics Department, CH Tours-Bretonneau University Hospital, Tours, France.

Background: The 22q13.3 deletion syndrome (MIM 606232) is characterized by developmental delay, absent or severely delayed speech, autistic behavior, normal to accelerated growth, and minor dysmorphic facial features. Among the three genes of the minimal critical region (from centromere to telomere: *SHANK3*, *ACR* and *RABL2B*), *SHANK3* is considered to be at the origin of the neurobehavioral symptoms. **Objective:** We describe the molecular characterization of a *de novo* interstitial del(22)(q13.3q13.3) disrupting the *SHANK3* gene in a child with a phenotype compatible with the deletion 22q13.3 syndrome. **Methods:** Clinical work up included clinical histories, physical, neurological, and ophthalmologic examinations, and imaging of the brain. Commercially available MLPA for subtelomeric analysis, FISH and quantitative real time PCR were used to characterize the rearrangement. **Results:** Subtelomeres analysis by MLPA showed a discrepancy between the P036B and P070 kits (MCR Holland): P070 MLPA probe (targeting *ARSA* gene) showed a deletion but P036B (targeting *RABL2B* gene) showed a normal result. FISH analysis using LSI TUPLE1/LSI ARSA (Vysis) probes confirmed deletion of *ARSA*, whereas FISH with N25/N85A3 (Cytocell) probes, targeting *SHANK3* locus was normal. Supplemented FISH analysis using BAC clones allowed to specify the centromeric breakpoint region of the interstitial deletion at less than 2Mb from the telomere. Quantitative real time PCR of exon 5, 22 and 24 and intron 9 of *SHANK3* showed the telomeric breakpoint occurred between intron 9 and exon 22. **Conclusions:** These data highlight the difficulty of performing an appropriate test aimed at looking for cryptic 22q13.3 deletion. Furthermore the molecular characterization of this interstitial 22q13.3 deletion contributes to clinical and genetic delineation of the 22q13.3 deletion syndrome.

Patterns of genetic differentiation between Mexican-Americans and three HapMap ethnic populations in candidate genes for depressive disorder and antidepressant response. *C. Dong, M.-L. Wong, J. Licinio* Dept Psychiatry, Univ of Miami, Miami, FL.

While major depressive disorder (MDD) is one of the most common diseases, the basis for ethnic differences in MDD susceptibility or antidepressant response is not well understood. To investigate the patterns of population differentiation of candidate genes for MDD and antidepressant response, we compared the allele frequencies of a panel of 956 SNPs in 270 candidate genes with genotypes in all four ethnic samples: 320 healthy Mexican-Americans (MA) recruited in our genetic study and three HapMap samples (CEU, CHB+JPT, YRI). F_{st} values were used to assess the population differentiation between Mexican-Americans and each of the three HapMap ethnic groups. Biological processes were determined on the basis of PANTHER classification. Overall, the greatest F_{st} was found between MA and YRI with an average value of 0.150, compared to 0.066 between MA and CHB+JPT and 0.043 between MA and CEU. The similar patterns were found by minor allele frequency, SNP type and biological process classification. General linear regression analysis showed a significant variation of F_{st} for population ($F_{(2, 3637)} = 152.84$, p 0.0001), minor allele frequency ($F_{(4, 3637)} = 19.04$, p 0.0001, SNP type ($F_{(3, 3637)} = 4.05$, p=0.0069) and biological process classification ($F_{(7, 3637)} = 3.01$, p=0.0037). A greater differentiation was also observed for coding SNPs (mean F_{st}=0.109), compared to intronic variants (mean F_{st}=0.060), in the genes in biological process of proteolysis. Our findings suggest a greater genetic differentiation between Mexican-Americans and Africans and a smaller differentiation between Mexican-Americans and Caucasians, and provide a possible basis for understanding of the genetic differentiation in susceptibility to MDD and antidepressant response across ethnic groups.

Identification of candidate transcription factors involved in human obesity. *J. Lane[1], L. Parnell[1], JP. Fortin[2], A. Kopin[2], J. Ordovas[1]* 1) Nutrition & Genomics, Tufts Univ, Boston, MA; 2) Molecular Cardiology Research Institute, Tufts Medical Center, Boston, MA.

Obesity is a complex disease that involves multiple tissues. Research in human populations is especially difficult, because of gene-by-environment interactions. We show that starting with data from a fat storage candidate gene screen in C.elegans, a novel bioinformatics approach can be used to generate candidate transcription factors (TFs) involved in obesity. In brief, human homologs of genes involved in fat storage in C.Elegans were identified. The homologs were grouped according to function and biological process, based on evidence that TFs can control groups of genes with similar function. To determine if the genes in each functional group are coordinately regulated, the upstream region of each gene in the group was scanned for common transcription factor binding sites (TFBS). TFBS that occurred in genes from the groups, but not in random sets of genes are candidates. The transcription factors (TFs) that bound to the TFBS of interest then became candidate TFs for involvement in obesity. The 46 mouse, rat, and human TFs identified as candidates were validated by searching databases of mouse knock-out data, quantitative trait loci, literature, and microarray data for evidence linking each TF back to obesity. The results highlights several promising candidates, such as CEBPB, ESR1, STAT5A, and THRB. The evidence also suggests the need for an in vitro validation of the candidates to assist in adjusting parameters in the bioinformatics screen. As an in vitro validation, during differentiation of mouse preadipocyte OP9 cells into adipocytes, measures of changes in gene expression were taken. The 46 genes will be measured for expression changes during adipocyte differentiation using a mouse microarray. Candidate transcription factors that show changes in expression level during adipocyte differentiation will be examined for single nucleotide polymorphisms (SNPs) that associate with obesity in human populations. This approach demonstrates that one can gain insight into complex diseases, such as obesity, by using publicly available data from model organisms.

Mutation analysis of the PMP22 and MPZ genes in Chinese patients with Charcot-Marie-Tooth disease. *W. Luo, Z. Ouyang* Neurology, Second Affiliated Hospital, Zhejiang University, Hangzhou, zhejiang, China.

Objective: To establish a technical platform in peripheral myelin protein 22 (PMP22) and myelin protein zero (MPZ) genes diagnosis by combining PCR based polymorphic short tandem repeats (STR) with denaturing high-performance liquid chromatography (DHPLC), and to detect the mutation of PMP22 and MPZ genes in 23 probands of Chinese patients with Charcot-Marie-Tooth disease (CMT) by using DHPLC. Methods: We detected the duplication mutation of PMP22 by PCR based polymorphic short tandem repeats, the PMP22 and MPZ point mutation by DHPLC in patients without PMP22 duplication mutation. The abnormal amplifications detected by DHPLC were sequenced. Further analysis was made to investigate whether the base variation was a polymorphism or a pathogenic mutation. Results: Seven cases of 23 CMT patients had PMP22 duplication analysis (30.4%). By using DHPLC to the mutation analysis of ten coding exons of the PMP22 and MPZ, we found eleven abnormal peak forms in nine patients. There were two in exon 4, 5 of MPZ, two in exon 5, 6 of MPZ, seven in exon 4 of PMP22. Furthermore, the amplifications with abnormal peak form were sequenced. We found two synonymous mutations in MPZ which were 657GA (Gly219Gly) in exon 5, 511AG (Pro171Pro) in exon 4, and one basic changes in the intron of PMP22 IVS4+32(CT)in seven patients. Conclusion: Established a technical platform in PMP22 and MPZ genes mutation analysis by combining PCR based polymorphic STR with DHPLC. Established the technology for mutation analysis of PMP22 and MPZ by DHPLC first in China. PCR based polymorphic STR can be used as a primary screening in the detection of PMP22 duplication analysis. Discovered a new synonymous mutations in MPZ, 511AG (Pro171Pro). Preliminarily test the reliability for the mutation analysis by DHPLC in PMP22 and MPZ. This study was funded by the grants from the National Natural Science Foundation of China (Grant No. 30672264), Health Bureau of Zhejiang Province(Grant No. 2005QN005) and Pao Yu-kong and Pao Zhao-long Scholarship.

Genomic organization of human satellite. *R. Ennesser[1], S. McCutcheon[2], M. Cummings[2], J. Doering[1]* 1) Dept. of Biology, Loyola Univ. Chicago; 2) Dept. of Bio. Sci., Univ. of Illinois at Chicago.

Beta satellite is a family of tandemly repeated 68-69 bp monomers located throughout the genome, including a 6.2 kb cluster distal to the FSHD-associated D4Z4 3.3 kb repeat array at 4q35. satellite clusters may be composed exclusively of monomers and exhibit a higher order repeat (HOR) structure or contain interspersed sequences similar to 3.3 kb repeats, which we have termed core. In order to elucidate the organization of satellite and core elements in the genome, we searched the NCBI database for core using sequence obtained from an isolated HC21p core element as well as for satellite using a sequence reported to be part of an HOR. We found both core and satellite on many chromosomes, but interestingly only two of the clusters are not associated with core, and both lacked any HOR. This finding suggests the NCBI database is underrepresented for satellite arrays. All 44 core sequences present in the NCBI database were examined and most were found to be 2.8 kb, but smaller, truncated copies also exist. The core sequences have only an 85-87% sequence similarity to each other as well to D4Z4 3.3 kb repeats. Sequence analysis identified four types of core-like element organizations, including 3.3 kb repeat arrays, classic elements flanked on both sides by satellite, junction elements flanked on only one side by satellite, and island elements not associated with satellite. The 3.3 kb arrays can also be subdivided into three groups based on distal organization. Analysis of satellite clusters flanking core has revealed high sequence similarity to regions of the 6.2 kb satellite cluster distal to 3.3 kb repeat arrays. We propose a model in which 2.8 kb core elements, including full-length LSau domains, were formed from D4Z4 via two recombinational steps with a third step producing truncations and resulting in the observed complex genomic organization. This study has provided the first high-resolution analysis of satellite and interspersed core sequences and clarified their organization at many loci.

Detection of copy number variation in mental retardation using high-density arrays. *P. Fortina[1,2], V. Alesi[1,3], L. Bernardini[3], S. Loddo[3], A. Novelli[3], I. Bottillo[3], A. Battaglia[4], M. C. Digilio[5], S. Surrey[6], B. Dallapiccola[2,3]* 1) Department of Cancer Biology, Thomas Jefferson University, Jefferson Medical College, Philadelphia, PA; 2) Department of Experimental Medicine, University La Sapienza, Rome, Italy; 3) CSS Hospital, IRCSS, San Giovanni Rotondo and CSS-Mendel Institute, Roma Italy; 4) IRCCS Istituto Scientifico per la Neuropsichiatria dell'Infanzia e dell'Adolescenza Stella Maris, Pisa, Italy; 5) Ospedale Pediatrico Bambino Gesù, Rome, Italy; 6) Cardeza Foundation for Hematological Research, Thomas Jefferson University, Jefferson Medical College, Philadelphia, PA.

Forty patients with moderate to severe mental retardation with at least one major malformation and/or dysmorphism and/or multiple minor anomalies were analyzed for pathogenic genomic changes using an oligonucleotide array with ~75 Kb spacing (Agilent Human Genome CGH 44K array), and a SNP array with ~5 Kb spacing (Affymetrix GeneChip 250K Nsp array). Copy number variations (CNVs) ranging from 0.2-22.5 Mb were identified in 11 patients (27.5%) using the Agilent array while CNVs from 0.1-23.0 Mb were identified in 16 different patients with the SNP array. CNVs were confirmed using FISH or qPCR, and 14 overlapped (74%) comparing oligonucleotide and SNP data. In both analyses, 8 patients had a deletion, 1 two non-contiguous duplications on the same chromosome, and 2 had a deletion and duplication on a single chromosome. The CNVs are presumed to be causally related to the phenotypes because of their extent and the genes involved. The SNP array showed 5 additional CNVs, including 3 deletions and 2 duplications ranging in size from 0.152-0.393 Mb confirming the higher density array is more sensitive in detecting smaller changes. An additional 32 patients with CNVs identified by other techniques were typed on the 250K Nsp, and showed agreement with previous results. The SNP array detected all changes identified by other techniques as well as a 9.5 Mb duplication not previously known. In summary, array-based platforms facilitate detection of CNVs but results must be confirmed independently and software settings must used to reduce false positives and identification of CNVs devoid of a pathogenic role.

Complement Factor H (CFH) and ARMS2/LOC387715 as Risk Factors for Age-Related Macular Degeneration in the French-Canadian Population. *M. Lanthier-Veilleux[1], P. Belleau[1], E. Deilhes[1], R. Arseneault[1], S. Dubois[1], M. Malenfant[3], N. Gaudreault[2], M.-A. Rodrigue[2], V. Raymond[1,2]* 1) Ocular Genetics & Genomic, CRCHUL, Québec City, QC, Canada; 2) Genomes Sequencing & Genotyping Platform, CRCHUL, QC, Canada; 3) Ophthalmology, Laval University Hospital (CHUL), QC, Canada.

Age-related macular degeneration (AMD), a leading cause of blindness, is a genetically complex disorder. In several Caucasian populations, variations in the genes of the alternate complement cascade represent major risk factors for AMD. However, this contribution still remains to be established in French-Canadians. To assess these genes on susceptibility to AMD in French-Canadians patients, we tested if SNPs in CFH, ARMS2/LOC387715, C-reactive protein pentraxin-related (CRP) or complement factor B (CFB)/complement component 2 (C2) were associated with the disorder. 111 unrelated FC patients and 113 controls matched for age and sex were genotyped using DNA sequencing or Sequenom mass array. AMD diagnosis was made according to the International Age-Related Maculopathy Epidemiological Study. PLINK was used to reconstruct haplotypes, Armitage tests to estimate associations. Within CFH, 2 SNPs in complete LD, rs10465586 and rs1410996, showed strong association with AMD. When carried in the TA homozygotic state, these 2 SNPs decreased by 3 fold risk for AMD. Five CFH haplotypes (17 SNPs) displayed a prevalence > 5 %. Two of these were strongly associated with AMD. The QCAMD1 haplotype was associated with an increased risk for the disorder and encompassed Y402H. On the other hand, the QCAMD2 haplotype, harboring H402H, decreased this risk. The 3rd haplotype, QCAMD3, was the only one containing the G allele at rs419137. Although it carried Y402H, its prevalence was similar between patients and controls. Among ARMS2 haplotypes, only the 1 harboring A69S increased risk for AMD. Variations in CRP and CFB/C2 were not associated with the disorder. This study demonstrates that variants of the CFH and ARMS2 genes represent major risk factors for AMD in French-Canadians. It is the 1st study to extend previous findings to 1 of the several Canadian populations.

Californias experience with a statewide prenatal screening program for chromosomal abnormalities. *M. Roberson, B. Currier, A. Davis, L. Malm, S. Smith, S. Riggle, C. Hodgkinson, C. Tempelis, L. Jellife-Pawlowski, L. Walton-Haynes, F. Lorey, N. Kazerouni* Genetic Disease Screening Program, California Department of Public Health, Richmond, CA.

Aim: Examine screening performance of Californias triple-marker screening program, utilizing data from a statewide registry for chromosomal defects. **Methods:** A total of 378,751 women who had successful screening and had an expected date of delivery in 2006 are included in the study. Follow-up diagnostic services for screen positive women were performed at State-approved centers. Data on diagnostic outcomes from these visits were entered into the statewide registry. Other registry sources include mandatory reporting by all cytogenetic labs, hospitals, State newborn screening records, and outcome data forms submitted by prenatal care providers. **Results:** The observed detection rate (DR) for Down syndrome (N=601) was 77%. It varied significantly by gestational dating method and maternal age. The rates for women under 35, and 35 and over were 61% and 95%, respectively. The DRs were 80% for ultrasound dated pregnancies and 69% for LMP-dated pregnancies. For Turners, trisomy 18, triploidy and trisomy 13, the DRs were 75%, 83%, 96%, and 39%, respectively. The Programs cut-off was a risk of 1:190 or greater for Down syndrome (DS) and 1:100 for trisomy 18 at mid-trimester, resulting in an overall program screen positive rate of 6.5%. The positive rate for DS was 5.3%. Of the women with a DS fetus that were screen positive, only 59% chose to have amniocentesis. Of the women who obtained results from amniocentesis indicating a DS fetus, 65.8 % had an elective termination, 22.4% had a live birth, 5.1 % had a demise or miscarriage, and 6.6% had an unknown outcome. **Conclusions:** The observed performance of this large triple-marker screening program exceeds generally predicted detection rates for DS. California added inhibin to its screening program in July of 2007 and will be adding first trimester markers in January of 2009. This study methodology will be used to measure the performance of these subsequent screening enhancements in this large interventional program.

Two cases of Mucolipidosis II presenting in the newborn period as a skeletal dysplasia consistent with Pacman Dysplasia. *J. Lester[1,3], T. Del Moral[2], M. Buch[2], M. Diaz-Barbosa[2], M. Fajardo[2], K. Wierenga[3], P. Jayakar[1,3], D. Barbouth[3], S. Sacharow[3]* 1) Medical Genetics, Miami Children's Hospital, Miami, FL; 2) Division of Neonatology, University of Miami, Miami, FL; 3) Division of Medical Genetics, University of Miami, Miami, FL.

Pacman dysplasia is a rare skeletal dysplasia with epiphyseal stippling and osteoclast overactivity. Mucolipidosis II (ML-II) is a condition caused by abnormal lysosomal enzyme transport due to N-acetylglucosamine-1-phosphotransferase deficiency, and results in multi-organ dysfunction, skeletal disease and cognitive decline. The proposition that Pacman dysplasia and ML-II are allelic has been controversial. We report two patients with skeletal dysplasia, consistent with Pacman dysplasia, diagnosed with ML-II by lysosomal studies. Baby A, a Hispanic male, was born to consanguineous parents at 39 weeks gestation by caesarean section because of failure to dilate. Birth weight was 2.5kg, length 46cm, and head circumference 32.5cm. At birth he had rhizomelic shortening, severe demineralization with fractures of several ribs, humeri, tibiae, and fibulae, and hypoplasia of the pelvis, rib shortening and a C7 compression fracture. Punctate calcifications of calcanei were noted. Physical exam showed micrognathia and hypertrophic gums. He required tracheostomy and gastrostomy placement. Baby B, an African-American male, was born by normal vaginal delivery at 30 weeks gestation. Birth weight was 1.5kg, length 39.5cm, and head circumference 28cm. At birth he had severe demineralization with fractures of several ribs, humeri, femora, tibiae and fibulae. Punctate calcifications of the calcanei and orbital bones were noted. Exam revealed a small mouth, contractures involving several fingers and toes, and hypertrophic gums. Since tracheal intubation, he has remained ventilator-dependent. Parathyroid hormone was elevated in both patients. Lysosomal enzymes showed high elevations of several enzymes in plasma with normal enzyme levels in leukocytes confirming the diagnosis of ML-II in both patients. These two cases add to the increasing body of evidence that Pacman dysplasia represents the severe end of the spectrum of ML-II.

Mobile elements create copy number variation in an individual human genome. *J. Xing[1], Y. Zhang[1], S. Levy[2], E. F. Kirkness[2], M. A. Batzer[3], L. B. Jorde[1]* 1) Dept Human Genetics, Univ of Utah, Salt Lake City, UT; 2) J. Craig Venter Institute, Rockville, MD; 3) Dept of Biological Sciences, Louisiana State University, Baton Rouge, LA.

Copy number variation has been the subject of much recent research. Because approximately half of the human genome consists of mobile, repetitive DNA sequences such as Alu and LINE1 elements, it is plausible that these elements play an important role in generating copy number variation. Sequencing of the diploid genome of one individual human (HuRef) affords us the opportunity to assess, for the first time, the impact of mobile elements on copy number variation in a thorough and unbiased fashion. In this study, we systematically examined more than 720,000 indels between the HuRef assembly and the Human Genome reference sequence. Combining computational analysis and PCR experiments, we identified and validated more than 600 mobile element insertion events (including Alu, L1 and SVA elements), which added ~270Kb new DNA sequence to the HuRef genome compared to the Human Genome reference sequence. We also identified more than 150 mobile element recombination-mediated deletions (RMDs) in the HuRef sequence. These events removed ~200Kb sequence from the HuRef genome. About half of the insertion/deletion events occurred in genic regions, and at least one deletion removed part of coding region of a predicted human gene. Most of these insertion/deletion events are polymorphic in human populations and have a higher frequency in European populations, in agreement with the HuRef donors ancestry. This study presents the first comprehensive analysis of mobile element variation in the complete DNA sequence of an individual and demonstrates that mobile elements play an important role in generating inter-individual indel variation. In addition, newly identified polymorphic mobile element insertions in the HuRef genome are useful tools for human population genetic studies.

A *SOD1* missense mutation in dogs with degenerative myelopathy: a spontaneous amyotrophic lateral sclerosis model. *G. S. Johnson[1], T. Awano[1], C. M. Wade[2,3], M. L. Katz[1,4], G. C. Johnson[1], J. F. Taylor[5], M. Perloski[2], T. Biagi[2], S. Long[6], S. Khan[1], D. P. O'Brien[7], K. Lindblad-Toh[2,8], J. R. Coates[7]* 1) Dept. of Veterinary Pathobiology, Univ. Missouri, Columbia, MO; 2) Broad Institute of Harvard and MIT, Cambridge, MA; 3) Center for Human Genetic Research, Massachusetts General Hospital, Boston MA; 4) Mason Eye Institute, Univ. Missouri, Columbia, MO; 5) Division of Animal Sciences, Univ. Missouri, Columbia, MO; 6) Section of Neurology and Neurosurgery, Univ. Pennsylvania, Philadelphia PA; 7) Dept. of Veterinary Medicine and Surgery, Univ. Missouri, Columbia, MO; 8) Dept. of Medical Biochemistry and Microbiology, Uppsala Univ., Sweden.

Canine degenerative myelopathy (DM) is a fatal neurodegenerative disease prevalent in several dog breeds. The initial signs usually occur at 8 years of age or older and are characterized by progressive upper motor neuron pelvic limb paresis and ataxia. If euthanasia is delayed, the clinical signs will ascend causing flaccid quadraparesis and other lower motor neuron signs. DNA samples from 38 DM-affected Pembroke Welsh corgi cases and 17 related clinically normal controls were used for genome-wide association mapping which produced the strongest association with markers on CFA31 in a region containing the canine *SOD1* gene. *SOD1* was considered a regional candidate gene because mutations in human *SOD1* can cause amyotrophic lateral sclerosis (ALS), an adult-onset fatal paralytic neurodegenerative disease with both upper and lower motor neuron involvement. Resequencing of *SOD1* in normal and affected dogs revealed a G to A transition, resulting in an E40K missense mutation. Homozygosity for the A allele was associated with degenerative myelopathy in five dog breeds ($p = 2.93E-19$). Microscopic examination of spinal cords from affected dogs revealed myelin and axon loss affecting the lateral white matter and neuronal cytoplasmic inclusions that bind anti-superoxide dismutase 1 antibodies. These inclusions are similar to those seen in spinal cord sections from ALS patients and transgenic rodents with *SOD1* mutations. Our findings identify canine DM to be the first recognized spontaneously occurring animal model for ALS.

Factors Impacting Transformation Efficiency of B Cells by Epstein Bar Virus in the NINDS Human Genetics DNA and Cell Line Repository. *R. A. Corriveau[1], K. P. Reeves[1], J. L. Andrews[1], C. M. Ziccardi[1], M. A. Keller[1], K. Gwinn[2], L. A. Mamounas[2], R. Zhang[2], C. M. Beiswanger[1], A. B. Ansbach[1]* 1) Coriell Institute for Medical Research, Camden, NJ; 2) National Institute of Neurological Disorders and Stroke, Bethesda, MD.

The NINDS Human Genetics DNA and Cell Line Repository was established in 2002 to support discovery of genetic risk for complex neurological diseases. To date the NINDS Repository has banked biospecimens and associated clinical data from more than 22,000 unique individuals. Samples are from donors with amyotrophic lateral sclerosis (ALS) and other motor neuron diseases, epilepsy, Parkinsons disease and related disorders, ischemic stroke, hemorrhagic stroke, aneurysm, and Tourettes syndrome, as well as from control individuals. To create a renewable biospecimen resource, B lymphocytes obtained from gradient-separated peripheral blood mononuclear cells are transformed with Epstein Barr virus (EBV) to establish lymphoblastoid cell lines. Here we evaluate the efficiency of B cell transformation by EBV. Variables evaluated include: age of subject, race, ethnicity, gender, diagnosis, smoking status, volume of blood collected, time between blood collection and processing, and type and concentration of anticoagulant. For ALS, we also analyze the relationship between disease treatment and transformation efficiency (riluzole versus no treatment). Preliminary data indicate that for 8.5 ml ACD blood collection tubes, blood specimens with volumes less than 4 ml or time to processing of more than eight days have decreased incidence of transformation. Additionally, samples submitted from younger subjects are more likely to transform successfully than samples from older subjects, with the exception of the very oldest subjects (over 90 years old), which display transformation efficiencies comparable to subjects aged 46 to 50 years (~94%). Finally, samples from subjects with the neurological diseases banked by the NINDS Repository do not appear to differ in their transformation efficiency as compared to control subjects.

Shorter telomeres in metaphase, interphase, and individual chromosomes 21, 1, 2, and 16, may indicate dementia and mild cognitive impairment in older adults with Down syndrome. *E. Jenkins[1], L. Ye[1], H. Gu[1], S. Ni[1], M. Velinov[1], D. Pang[1,2], S. Krinsky-McHale[1], W. Zigman[1], N. Schupf[1,2], W. Silverman[1,3]* 1) Dept. Hum. Genet., NYS Inst. for Basic Res. in Development. Disabil., Staten Island, NY; 2) Taub Inst. for Res. on Alz. Disease and the Aging Brain, Columbia University, 630 West 168th St., New York, NY; 3) The Kennedy Krieger Inst. and The Johns Hopkins Univ. School of Medicine, Suite 222s, 707 Broadway, Baltimore MD.

We have reported shorter telomeres (indicated by reduced fluorescence light intensities from a PNA telomere probe) in T lymphocytes of older adults with Down syndrome (DS) and dementia due to Alzheimers disease (AD; Jenkins et al., 2006). We have now extended that study by discovering that adults with DS and mild cognitive impairment (MCI-DS) also have shorter telomeres than adults with DS without MCI-DS. Cumulatively, these findings have been observed in 15 older adults with DS and dementia compared to 15 adults with DS without dementia and 8 adults with MCI-DS compared to 8 age-matched adults with DS without MCI. Additionally, new findings have shown that fluorescence light intensity measurements from chromosome 21 alone, or in combination with chromosomes 1, 2, and 16, exhibited reduced light intensities in telomeres from adults with DS with either dementia or MCI-DS compared to non-demented adults with DS only. Chromosome 21 measurements appear to be promising for use as a biomarker because there was no overlap in light intensities between demented or MCI-DS and non-demented participants with DS without MCI. Since early clinical symptoms of AD can be very difficult to recognize in this population of adults due to their pre-existing cognitive impairments, a valid biomarker would be of great value. Early detection of AD is especially important because it would allow treatments to begin before the occurrence of significant damage to the central nervous system. Our findings suggest the feasibility of using telomere shortening as a biomarker for accurately indicating dementia status. Supported in part by NYS OMRDD, Alz. Assoc. grants IIRG-07-60558, IIRG-99-1598 and IIRG-96-077; NIH grants PO1-HD35897, RO1-HD37425, and RO1-AG014673.

Screening Small Molecules for Rescue of Peroxisome Assembly Defects. *N. Braverman[1], R. Zhang[2], S. Steinberg[3]* 1) Dept Human Gen & Pediatrics, McGill Univ, MCH Res Inst, Montreal, PQ, Canada; 2) McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins Medical Ctr, Baltimore MD; 3) Dept Neurogenetics, Kennedy Krieger Institute, Baltimore, MD.

Zellweger spectrum disorder (ZSD) is a heterogeneous group of autosomal recessive diseases with high morbidity and mortality caused by a failure to assemble normal peroxisomes. There are no therapies for ZSD and management is supportive. Nevertheless about half of the patients have a phenotype milder than classic Zellweger syndrome and exhibit a progressive disease course. Thus patients would benefit if therapies become available and are instituted early. Recent observations suggest that improving the conformation of a defective Pex protein, increasing its expression, or increasing peroxisome numbers results in partial recovery of peroxisome function in ZSD fibroblasts. We utilized a patient cell line with a common genotype, containing the *PEX1*-Gly843Asp and Ile700fs alleles, and engineered this to over-express a GFP-tagged PTS1 matrix protein that remains cytosolic at baseline. 2400 compounds from two small molecule libraries containing known bioactive chemicals were screened first. The cells were cultured in each compound in duplicate for two days, fixed and imaged using epifluorescent microscopy on a high content imaging platform. Redistribution of the GFP tagged matrix protein from the cytosol to the peroxisome membranes was scored visually and compared to both positive and negative controls. Currently we have identified four compounds that recover peroxisome matrix protein import and confirmatory studies are in progress. Initial studies indicate that *PEX1*-G843D homozygous and compound heterozygous primary fibroblasts attain similar functional improvement. This will be a robust platform for drug identification because analysis is based on recovery of downstream function, and is not biased toward mechanism.

Characterization and Linkage Analysis of a Large Amish Family with an Increased Prevalence of Multiple Sclerosis and Hodgkins Lymphoma. *C. A. Gurnett[1], L. Kruse[2], D. M. Desruisseau[1], W. J. Linz[3], C. E. Jackson[3]* 1) Dept Neurology, Washington University, St Louis, MO; 2) Washington University School of Medicine, St Louis, MO; 3) Scott and White Hospital, Temple, TX.

Multiple sclerosis (MS) is a multifactorial neurological disorder that is influenced by complex interactions between genetic and environmental factors. Recent genome wide association studies have identified numerous risk factors associated with MS though these only explain a small proportion of the risk for disease. We identified a four-generation Amish family from Daviess County, Indiana that consisted of ten individuals with multiple sclerosis (nine females, one male). Eight were diagnosed with clinically definite and two with clinically probable multiple sclerosis. The phenotype varied with regard to age of onset (20-50 yr) and clinical course (six relapsing remitting, two secondary progressive, and two primary progressive). Included in the family is a pair of identical twins concordant with similar clinical course (relapsing-remitting) and age of onset (23 and 30 yr). There were also four closely related males with Hodgkins lymphoma. All but one individual with multiple sclerosis had at least one copy of the HLA DRB1*1501 high-risk allele (rs3135388 A allele). Affymetrix 250K StyI genotype data were obtained and genome wide linkage analysis was performed with GENEHUNTER. Three chromosomal regions were identified with NPL scores >4 (11p14.2, NPL=7.86; 1p36.22, NPL=4.91; 6p22, NPL=4.87). Haplotype analysis enabled mapping of these regions to 6.5 cM on chromosome 1p36.22 and 3 cM on chromosome 11p14.2; the region on chr 6p22 contains HLA-DRB1. These regions, along with the high-risk HLA DRB1*1501 polymorphism, were a strong predictor of disease in this cohort. Resequencing of candidate genes (TNFRSF9, TNFRSF25, RPL22, KLHL21, and BDNF) in these regions of chromosome 1 and 11 did not reveal any novel mutations. This study identified two loci that, when inherited with HLA DRB1*1501, are a strong predictor of disease presence in this Indiana Amish family.

Further evidence of modifier gene(s) for glaucoma. *P. Belleau[1], S. Dubois[1], R. Arseneault[1], J. L. Anctil[2], E. Shink[1], M. A. Rodrigue[1], G. Côté[2], M. Amyot[3], V. Raymond[1,2], The Québec Glaucoma Network* 1) Ocular Genetics & Genomics, CREMO, Laval University Hospital (CHUL) Res Ctr, Québec City, PQ, Canada; 2) Ophthalmology, Laval Univ, Québec City; 3) Ophthalmology, Univ of Montréal, Montréal, PQ, Canada.

Primary open-angle glaucoma (POAG) is a complex disorder characterized by an optic neuropathy and visual field loss. We reported 2 huge autosomal dominant glaucoma families in which the deleterious genes were associated with wide phenotypic variabilities. In the CA family, POAG was caused by the $MYOC^{K423E}$ mutation while, in the BV kindred, the disorder was due to a *FOXC1* duplication. In the CA pedigree, $MYOC^{K423E}$ carriers clustered into large groups with extreme ages at onset (AAO). This finding supported the presence of 1 modifier altering glaucoma severity. To investigate if this variability in the BV pedigree was also caused by modifier gene(s) interacting with the primary mutation, we assessed if sub-phenotypes for the disorder clustered in particular branches of the kindred. Of the 201 BV individuals studied, 41 were carriers of the *FOXC1* duplication. Among those, 20 carriers were POAG, 12 were suspects and 9 were asymptomatics. AAO varied from 5 to 75 years old in these carriers with a mean of 34 years of age. Clusters were built using a neighboring approach in which the neighborhood of each carrier was defined as a set of pedigree members in which the kingship coefficient between the carrier and other members was 0.0625. For each carrier of the duplication, we next calculated the median of AAO for his/her neighborhood and each carrier was classified into 1 of 3 groups according to this median: group 1, 25 years old; group 2, between 26 and 38; and group 3, 39. Using this approach, 5 distinct clusters were detected in the BV pedigree; 3 were in group 3 while the other 2 clusters were in the group 1. In conclusion, in the BV family, carriers of the *FOXC1* duplication clustered into 5 large groups with extreme ages at onset for glaucoma. This observation confirms our previous finding of the presence of modifier gene(s) that alters glaucoma severity when the disorder is caused by a primary mutation.

Cardio-facio-cutaneous syndrome (CFCS) due to a novel MEK2 mutation involving four generations: Clinical delineation and natural history. *Y. Lacassie[1], S. Sampath[2], H. M. Peltier[3], S. Bale[4], K. A. Rauen[5]* 1) Dept Ped/Div Clin Genetics, LSUHSC and Children's Hospital, New Orleans, LA; 2) Dept of Genetics, LSUHSC, New Orleans; 3) The Ctr for Pediatric and Adolescent Medicine, Thibodaux, LA; 4) GeneDx, Gaithersburg, MD; 5) Div of Medical Genetics, Dept of Pediatrics, San Francisco, CA.

CFCS is a rare AD disorder characterized by cardiac abnormalities, distinctive craniofacial appearance and ectodermal anomalies. Learning disability, short stature and other findings have been reported. All cases reported are due to *de novo* mutations mainly in the BRAF but also in the MEK1, MEK2 and KRAS genes. We report 9 affected relatives spanning 4 generations detected through the diagnosis of a 7½ mo WM referred for evaluation of short stature and pulmonic stenosis (PS). The proband had a history of pyloric stenosis and had curly hair, a distinctive face with large forehead, absence of eyebrows and hypertelorism. A cavernous hemangioma, a CALS, penoscrotal inversion, ridge dysplasia and low TRC were also noted. His mother had a history of PS and learning difficulties and presented with short stature and similar CF features. From a previous union, mom had a 7-yo son with history of pyloric stenosis, lazy eye, inguinal hernia and ADHD, who on PE had prominent forehead, sparse, curly hair, absence of eyebrows, strabismus, pointed lateral incisors, a CALS, a hemangioma, pectus excavatum, minor penoscrotal inversion, a CPL in F4 and low TRC. In follow up at 1yo, along with an old family picture, it became evident that his condition was CFCS with 9 affected relatives involving 4 generations. Molecular testing showed a novel P128Q mutation in the MEK2 gene segregating with the disorder. The clinical evaluation of the family showed an evolving phenotype. All affected relatives had evident absence of the eyebrows, curly hair and prominent forehead. Most affected members had sparse hair. Learning disability and strabismus were common. PS was only seen in the proband and his mother. This large family allows the delineation of clinical features and natural history and has important implications in current genetic counseling.

Study of families with discordant/concordant sibling pairs for comgenitally missing teeth. *E. Severin[1], C. Albu[1], D. Stanciu[2], D. Albu[1]* 1) Dept Human Genetics, Carol Davila Univ Med Pharm, Bucharest, Romania; 2) Dept Orthodontics, Carol Davila Univ Med Pharm, Buharest, Romania.

Background: There is evidence that genetic factors control the phenotypic variation of dental number abnormalities and autosomal dominant forms of congenitally missing teeth are caused by different mutations in human MSX1, PAX9 and AXIN2 genes. Objective: The aim of the study is to ascertain the occurrence and characteristics of tooth agenesis in sibling pairs and to compare the findings in different families. Patients and Methods: Data from seven Caucasian sibling pairs with a family history of non-syndromic permanent missing teeth, ranging in age from 10 to 27 years, were used for analysis. Clinical, radiographic and genetic examinations were carried out. Results: Clinical examinations revealed five families in which one sibling of the sibling pair has tooth agenesis and the other has not and two families in which siblings are concordant for hypodontia but discordant for the pattern of congenitally missing teeth. There are three main groups of sibling pairs: female pairs, male pairs and female-male pairs. In all cases, one of the parents shows the clinical phenotype of hypodontia. Hypodontia is inherited as a dominant trait with autosomal transmission, complete penetrance and highly variable expression. Conclusions: Phenotypic evaluations have revealed that expression of tooth agenesis varies widely not only among, but also within families.A comparison of interfamilial and intrafamilial variability in expressivity found significantly greater interfamilial variability, suggesting that more than one gene or allele might be involved.

MAST3: a Novel IBD Risk Factor that Modulates TLR4 Signaling. *C. Labbe[1], P. Goyette[1], C. Lefebvre[1], C. Stevens[2], T. Green[2], M. K. Tello-Ruiz[2], Z. Cao[3], A. Landry[3], J. Stempak[4], V. Annese[5], A. Latiano[5], S. Brant[6], R. Duerr[7], K. Taylor[8], J. Cho[9], A. H. Steinhart[4], M. Daly[2], M. Silverberg[4], R. Xavier[3], J. D. Rioux[1]* 1) Université de Montréal, Montreal Heart Inst, Montreal, Canada; 2) The Broad Institute of MIT and Harvard, Cambridge, MA; 3) Center for Computational and Integrative Biology and Gastrointestinal Unit, MGH, Harvard, Boston, MA; 4) Mount Sinai Hospital IBD Center, U. of Toronto, Toronto, On, Canada; 5) Gastrointestinal and Endoscopy Units, I.R.C.C.S. Hospital, San Giovanni Rotondo, Italy; 6) Meyerhoff Inflammatory Disease center, Johns Hopkins University School of Medicine, Baltimore, MD; 7) School of Medicine, University of Pittsburgh, Pittsburgh, PA; 8) IBD Center, Cedars-Sinai Medical Center, Los Angeles, CA, USA; 9) IBD Center, Department of Medicine, Yale University, New Haven, CT.

Inflammatory bowel disease (IBD) is a chronic disorder caused by multiple factors in a genetically susceptible host. Significant advances in the study of genetic susceptibility have highlighted the importance of the innate immune system in this disease. We previously completed a genomewide linkage study and found a significant locus (IBD6) on chromosome 19p. We were interested in identifying the causal variant in IBD6. We performed a two-stage association mapping study. In stage one, 1530 SNPs were selected from the HapMap database and genotyped in 761 patients with IBD. Among the SNPs that passed the threshold for replication, 26 were successfully genotyped in 754 additional patients (stage two). One intronic variant, rs273506 located in the MAST3 gene was found to be associated in both stages (pooled $P=2x10^{-4}$). We identified four MAST3 coding variants, including a non-synonymous SNP rs8108738, correlated to rs273506 and associated to IBD. To test whether MAST3 was expressed in cells of interest, we performed expression assays which showed abundant expression of MAST3 in antigen presenting cells and in lymphocytes. The knockdown of MAST3 specifically decreased TLR4 dependent NF-B activity. Our findings are additional proof of the pivotal role played by modulators of NF-B activity in IBD pathogenesis.

Novel systems-based endophenotypes spanning psychosocial stress, hormones, and vascular damage for genome wide linkage and association analyses in the Jackson Heart Study. *S. G. Buxbaum[1], M. Sims[2], E. Fox[2], L. Ekunwe[1], K. T.Cuenco[3]* 1) Jackson Heart Study, Jackson State Univ, Jackson, MS; 2) Jackson Heart Study, School of Medicine, University of Mississippi, Jackson, MS; 3) School of Dentistry, University of Pittsburgh, Pittsburgh, PA.

Earlier studies have shown that the risk for accumulating vascular damage leading to disease is elevated in African Americans (AA), and that psychological stress and hormone levels may play an important role in influencing vascular outcomes. Using extensive pedigree, biologic, and psychosocial data in the Jackson Heart Study (JHS),a population-based study that has a primary goal of identifying underlying causes of AA vascular disease, we are conducting genomewide studies of novel endophenotypes that incorporate vascular, endocrine, and psychosocial measures. 2108 JHS AA(including 539 family members) have provided psychosocial stress and biologic data on hormones and inflammation in addition to vascular disease outcomes during 2004-8. Psychosocial stress levels were assessed by responses to questions regarding negative life events, feelings of hopelessness, negative societal interactions, anger, violence, social support, and SES. Hormone and endocrine measures included cortisol, insulin, endothelin, and aldosterone as well as reproductive history. Vascular damage was assessed through inflammation and cell adhesion measures. Factor analyses identified a system of correlated stress and vascular measures. Genomewide linkage analysis and family-based transmission disequilibrium tests for association were conducted on multiallelic markers. Preliminary results suggest that vascular and psychosocial stress are quantifiable using a system of five multivariate factor scores (heritability ranging from 0.25 to 0.51). Initial inkage peaks mapped to regions of chromosomes 1p34.1-p32 (p=7.9 x 10-5), 3p25.3 (p=3.7 x 10-6), 10p12 (p=1.2 x 10-6), and 15q12 (p=6.0 x 10-5). Single marker associations of nuclear families suggest that AVSD2 and EIG5 (susceptibilities to atrioventricular septal defect and idiopathic general epilepsy) may be correlated with systems-based vascular damage measures (p-values=0.01 and 0.044, respectively).

Novel Copy Number Variants in Children with Autism and Additional Developmental Anomalies. *L. Davis[1, 2], K. Meyer[1, 2], D. Rudd[1, 2], A. Librant[1], E. Epping[1], V. Sheffield[2, 3, 4], T. Wassink[1, 2]* 1) Dept Psychiatry, Univ Iowa, Iowa City, IA; 2) Interdepartmental Genetics Program; 3) Dept Pediactrics, Univ Iowa, Iowa City, IA; 4) Howard Hughes Medical Institute, Univ Iowa, Iowa City, IA.

Autism is a neurodevelopmental disorder characterized by three core symptom domains: ritualistic-repetitive behaviors, impaired social interaction, and impaired communication and language development. Recent studies have highlighted recurrent copy number changes in autism, such as 16p11.2 deletions and duplications (Christian et al. 2008; Marshall et al. 2008; Weiss et al. 2008), as well as a significant role for unique, novel variants (Christian et al. 2008; Davis et al. 2008; Roohi et al. 2008). We used Affymetrix 500K GeneChip Microarray technology to detect these smaller microdeletions and duplications in a subset of children from the Autism Genetic Resource Exchange (AGRE). In order to enrich our sample for potentially pathogenic CNVs we selected children with autism who had additional phenotypic features suggestive of a developmental disturbance (positive criteria filter) but who had normal cytogenetic testing (negative criteria filter). We identified families with the following features: two or more children with autism, at least one of whom also had facial dysmorphology, limb or digit abnormalities, or ocular abnormalities. To detect changes in copy number we used a publicly available program, Copy Number Analyser for GeneChip (CNAG) Ver. 2.0, developed at The University of Tokyo (Nannya et al. 2005). We identified novel deletions and duplications on chromosomes 1q24.2, 2q37.3, 3p26.1, 3p26.2, 4q34.2, and 6q24.3. Several of these deletions and duplications include new and interesting potential candidate genes for autism such as syntaxin binding protein 5 (STXBP5 also known as tomosyn) and leucine rich repeat neuronal 1 (LRRN1 also known as NLRR1). Lastly, our data suggest that rare pathogenic microdeletions and duplications may have a substantially higher prevalence in children with autism plus additional developmental anomalies.

Dysregulation of *p63* (*TP73L*) Expression and Desmosomal Assembly as Etiologic Factors for Bladder Exstrophy-Epispadias Complex. *B. Ching[1], G. Yagnik[1], L. Qi[2], C. Nauta[1], A. Hata[1], M. Ludwig[3], H. Reutter[4], C. Naydenov[5], J. P. Gearhart[6], S. A. Boyadjiev Boyd[1,6]* 1) Dept of Pediatrics, Section of Genetics, Univ California Davis, Sacramento, CA; 2) Rowe Program in Human Genetics, Dept Public Health Sciences, Univ California Davis, Davis, CA; 3) Dept of Clinical Chemistry and Pharmacology, University of Bonn, Bonn, Germany; 4) Dept of Human Genetics, University of Bonn, Bonn, Germany; 5) Dept of Chemistry and Biochemistry, Medical Univ Sofia, Sofia, Bulgaria; 6) Dept of Urology, The James Buchanan Brady Urological Institute, Johns Hopkins University, Baltimore, MD.

The bladder exstrophy-epispadias complex (BEEC) is a spectrum of congenital anomalies of the abdominal wall, bladder, bony pelvis, and genitalia, ranging from isolated epispadias, to its most severe form, cloacal exstrophy (OEIS complex). The etiology of BEEC is unknown, but there are indications that genetic factors contribute to this birth defect. Loss of function of *p63*, a *p53* homolog, causes developmental defects of skin and limbs in transgenic mice. Bladder exstrophy and related urogenital defects are also present in a proportion of the knockouts. Furthermore, *p63* gain of function mutations in humans cause at least 5 different genetic syndromes that present with urogenital anomalies. We have performed detailed *p63* sequencing and expression analysis in a cohort of BEEC patients. Although no obvious mutations have been detected thus far, we observed reproducible dysregulation of variable p63 isoforms in eight of fourteen blood- or bladder- derived cDNA samples. Independently, microarray expression analysis of normal vs. exstrophic bladder samples was carried out to identify differentially expressed genes. A higher than expected proportion of the differentially expressed genes were related to desmosomal assembly and function, suggesting an association of BEEC with desmosomal dysregulation. Interestingly, the downstream target of *p63*, *PERP*, encodes a tetraspan protein that is an integral part of the desmosome. Consistent with the suggested multifactorial inheritance of this birth defect, we suggest that *p63* defects lead to dysregulation of desmosomal assembly, thus contributing to the etiology of BEEC.

Reduced expression of integrin v8 is associated with brain arteriovenous malformation (BAVM) pathogenesis. *H. Kim[1,2], H. Su[1], L. Pawlikowska[1,2], H. Kitamura[3], F. Shen[1], S. Cambier[3], J. Markovics[3], M. T. Lawton[4], S. Sidney[5], A. Bollen[3], P.-Y. Kwok[2], L. Reichardt[6], W. L. Young[1,4], G.-Y. Yang[1,4], S. L. Nishimura[3]* 1) Dept Anesthesia, UCSF, San Francisco, CA; 2) Institute for Human Genetics, UCSF, San Francisco, CA; 3) Dept Anatomic Pathology, UCSF, San Francisco, CA; 4) Dept Neurological Surgery, UCSF, San Francisco, CA; 5) Division of Research, Kaiser Permanente Northern California, Oakland, CA; 6) Dept Physiology, UCSF, San Francisco, CA.

Background: Brain arteriovenous malformations (BAVMs) are a potentially devastating hemorrhagic disease with little known of their pathogenesis. TGF- signaling is required for proper vessel development, and defective TGF- signaling has been implicated in BAVM pathogenesis. We hypothesized that expression of the TGF- activating integrin, v8, might be reduced in BAVMs and that decreased 8 expression might lead to defective neoangiogenesis. **Methods:** We tested integrin v8 involvement in BAVM pathogenesis by: 1) determining 8 protein expression in perivascular astrocytes by immunostaining BAVM lesional tissue and controls, 2) measuring angiogenic response to focal vascular endothelial growth factor (VEGF) stimulation in adult brains of conditional itgb8 knock-out mice, 3) evaluating association of common genetic variants in ITGB8 with BAVM susceptibility in 194 BAVM cases and 127 healthy controls of Caucasian ancestry, and 4) correlating ITGB8 genotypes with 8 immunostaining in a subset of 28 patients. **Results:** Integrin 8 expression was significantly decreased in perivascular astrocytes of BAVM tissue compared to controls (P=0.002). Adenoviral Cre-mediated deletion of 8 in adult mouse brain resulted in enlarged dysplastic vessels in response to focal VEGF stimulation. Two ITGB8 SNPs (rs10486391 and rs11982847) and their haplotypes in the 5' region were associated with BAVM in Caucasians. BAVM-associated SNP genotypes were also associated with 60% decreased expression of integrin 8 in BAVM tissue (P=0.016). **Conclusion:** Reduced expression of integrin 8 is involved in the pathogenesis of sporadic BAVMs.

Development and evaluation of new mask protocols for gene expression profiling in humans and chimpanzees. *D. Toleno[1], G. Renaud[2], T. Wolfsberg[2], K. Siegmund[3], J. Hacia[1]* 1) Department of Biochemistry and Molecular Biology, University of Southern California, Los Angeles, CA, USA; 2) National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland, USA; 3) Department of Preventive Medicine, University of Southern California, Los Angeles, CA, USA.

Gene expression oligonucleotide microarrays are typically designed to detect the abundance of specific transcripts based on a reference genome sequence. Consequently, sequence differences between the arrayed oligonucleotide probes and transcriptome under consideration can lead to spurious estimates of transcript abundance. Here, we developed new methods for probe masking based on the most recent releases of human and chimpanzee genome sequences. Using publicly available tools from Bioconductor, data from probes predicted to have poor hybridization sensitivity and specificity to human and/or chimpanzee transcriptomes are discarded, followed by the removal of data from probe tilings with limited numbers of remaining probes. We investigated the effects of varying probe number on estimation of gene expression scores from five tissues derived from six humans and five chimpanzees. We consistently found that probe sampling has a significant effect on the variation of gene expression scores across samples, with fewer sampled probes resulting in more apparent expression variation for a probe tiling. The effect of probe number is greater when probe tilings have less than six probes remaining relative to the effect observed for gene expression estimated from between six probes and the full complement of eleven probes. Based on replicate probe sampling analyses, we found that the false positive and false negative rates for identifying cross-species gene expression difference increase with decreasing probe number. Overall, we provide a new resource for the analysis of human and chimpanzee transcriptomes and novel guidelines for probe masking.

Mutations in SPG11 and SPG15 account for the majority of cases with autosomal recessive complicated spastic paraplegia with thin corpus callosum. *G. Stevanin[1,15], C. Goizet[1,14], A. Boukhris[1,12], S. Hanein[1], P. Denora[1,13], H. Azzedine[1], P. Coutinho[2], A. Lossos[3], M. Tazir[4], A. Hamri[5], A. Benomar[6], M. Huntchinson[7], A. L. Rosa[8], M. Tada[9], J. Vale[10], C. Tallaksen[11], A. Durr[1,15], C. Mhiri[12], F. M. Santorelli[13], A. Brice[1,15], SPATAX network* 1) INSERM / UPMC U679 - NEB, Paris, France; 2) Hosp. S. Sebastiao, Santa Maria da Feira, Portugal; 3) Hadassah-Hebrew Univ. Med. Ctr., Jerusalem, Israel; 4) Mustapha Hosp., Algiers, Algeria; 5) Benbadis Hosp., Constantine, Algeria; 6) Specialities Hosp., Rabat, Morocco; 7) St Vincent's Univ. Hospital, Dublin, Ireland; 8) Sanatorio Allende, Cordoba, Argentina; 9) Niigata Univ., Japan; 10) De Egas Moniz Hosp., Lisboa, Portugal; 11) Ulleval Univ. Hosp., Oslo, Norway; 12) Habid Bourguiba Hosp., Sfax, Tunisia; 13) Bambino Gesu Hosp., Rome, Italy; 14) CHU Pellegrin, Bordeaux, France; 15) Dpt Genetics, Pitie-Salpetriere Hosp., Paris, France.

Autosomal recessive hereditary spastic paraplegias (ARHSP) with thin corpus callosum (TCC) are neurodegenerative diseases characterized by lower limb spasticity and mental impairment. Recently, we identified mutations in the SPG11 and SPG15 genes in patients. Their expression patterns are very similar in rat adult brain and expression is also detected in embryos. In a large series of index patients (n=127, 74 with AR-HSP, 53 sporadic cases) with this condition, we have identified 51 mutations in the SPG11 gene segregating in 111 cases (44 families) and in 17 apparently isolated cases, from 16 Countries. In SPG15, 16 different truncating mutations have been identified in 15 families (in progress). Overall, the phenotype of the SPG11 or SPG15 patients is severe and includes mental retardation or cognitive decline, lower motor neuron degeneration and slight cerebellar signs. In addition to TCC, brain MRI reveals frequently white matter alterations and cortical atrophy that worsen with disease duration. Disease onset occurs during the first to the third decade by problems with gait and/or mental impairment. Our study reveals the high frequency of SPG11 and SPG15 mutations in patients with HSP, TCC and mental/cognitive impairment, including isolated patients with this clinical profile.

A novel susceptibility locus for type 1 diabetes maps to human chromosome 21q22.3. *S. Onengut-Gumuscu[1], P. Concannon[1], J. A. Todd[2], D. J. Smyth[2], F. Pociot[3], R. Bergholdt[3], B. Akolkar[4], H. A. Erlich[5], J. E. Hilner[6], C. Julier[7], G. Morahan[8], J. Nerup[3], C. Nierras[9], WM. Chen[1], S. S. Rich[1], Type 1 Diabetes Genetics Consortium* 1) Ctr for Public Health Genomics, Univ of Virginia, VA, USA; 2) JDRF/WT DIL, Cambridge Inst for Medical Research, Univ of Cambridge, UK; 3) Steno Diabetes Ctr, Denmark; 4) Div of Diabetes, Endocrinology and Metabolic Diseases, NIDDK, NIH, MD, USA; 5) Roche Molecular Systems, CA, USA; 6) Wake Forest Univ, Health Sciences, NC, USA; 7) Inst de Genomique CNG, France; 8) WA Inst for Medical Research, Univ of Western Australia, Australia; 9) JDRF, NY, USA.

In an effort to map genomic regions linked to Type 1 diabetes (T1D), the Type 1 Diabetes Genetics Consortium (T1DGC) has genotyped 6,090 single nucleotide polymorphisms (SNPs) in 2,496 affected sib pair families. In the current study, we exploited the genome-wide linkage scan data to test for evidence of family-based association to T1D, using the pedigree disequilibrium test (PDT). P-values for each SNP were ranked, the top three SNPs localized to previously identified T1D risk loci INS (11p15), IFIH1 (2q24), and KIAA0350 (16p13). The fourth most strongly associated SNP is rs876498 ($P= 1.0\times10^{-4}$), which maps to 21q22.3, in a region that previously was not associated with T1D risk. rs876498 is located within the 'Ubiquitin associated and SH3 domain containing protein 3A' (UBASH3A) gene. UBASH3A is expressed primarily in lymphoid cells where it interacts with c-CBL and may help to down-regulate protein tyrosine kinases that are activated upon T-cell receptor stimulation. The association of T1D with rs876498 was replicated in two independent sets of samples, 2,214 trio T1D families from USA and Denmark and 17,400 British cases and controls. Overall the minor allele of rs876498 is significantly associated with T1D ($P= 6.4 \times 10^{-12}$; OR= 1.10; 95% CI[1.07-1.13]). This study provides compelling support for a novel T1D risk locus on chromosome 21, and suggests further genetic and functional studies are warranted to identify the causative variant(s) within this region.

BFAST: Blat-like fast alignment search tool. *N. Homer, S. F. Nelson, B. Merriman* University of California Los Angeles 5554 Gonda, 695 Young Drive South David Geffen School of Medicine at UCLA Los Angeles, CA 90095-7088.

Recently developed high-throughput next-generation sequencing technologies have begun to replace the previously dominant Sanger sequencing technology in terms of cost and throughput. Technologies like Illumina's Genome Analyzer, Roche's 454, and ABI's SOLiD are able sequence billions of bases in a matter of days. These technologies rely on producing short reads making the alignment of these reads to a reference genome difficult. Additionally, these technologies allow for paired-end reads, which further increases the capacity and also the potential accuracy when considered during alignment. Nevertheless, the number of reads generated by these high-throughput systems overwhelms current algorithms in the context of whole-genome sequencing. Additionally, these algorithms encounter difficulty when aligning reads that include sequence variants. Therefore we have developed a new sequence alignment tool for whole-genome sequencing called BFAST, or Blat-like Fast Alignment Search Tool. BFAST uses various subsets of bases for a given read to search corresponding index of the reference genome. In this fashion, the search space is significantly reduced giving a set of Candidate Alignment Locations (CALs) for the read, which can be subsequently aligned using standard dynamic programming techniques. BFAST is fully generalizable as to be robust against common variants such as mismatches, insertions and deletions as well as sequencing errors. We have implemented a multi-threaded and parallelizable software suite that can now be used to quickly and accurately perform whole-genome sequencing.

Identification of Autism candidate gene through proteomic profiling in Drosophila melanogaster heads. *S. Sarkardei[1], E. Cummings[2], G. Hilliard[2], L. Reiter[1]* 1) Neurology, UTHSC, Memphis, TN; 2) Department of Mol Sci, UTHSC, Memphis, TN.

Autism is an enigmatic childhood disorder of unknown origin, characterized by developmental, language and social deficits, ranging in severity from patients with profound deficits to individuals that are high functioning. Autism spectrum disorders (ASD) affect almost 1 in 150 children, males 3-4 times more than females, but in only 10% of these individuals is autism associated with a recognized cause. Understanding the molecular pathways dysregulated in Angelman syndrome (AS), a disorder related to autism, can provide key insights leading to identification of autism susceptibility genes and pathways. Approximately 3% of all autism cases result from maternal duplications of the region containing the AS gene *UBE3A*. The protein targets of *UBE3A* which cause the neurological defects observed in patients with AS are still largely unidentified. We have been using a novel strategy to identify these protein targets using over-expression and protein profiling in the brains of Drosophila melanogaster. In this approach we express high levels of both human and Drosophila *UBE3A* proteins in fly head using the GAL4/UAS system. To identify these potential protein targets, *UBE3A* is over-expressed in the brains of flies ubiquitously using the *Heatshock*-GAL4 driver. The targets are then identified by Rotofor-assisted proteomic profiling and followed by MALDI-ReTOF protein identification. We will discuss the neurologically relevant targets identified so far, which are involved in energy metabolism; dopamine biosynthesis; cell motility and neuromuscular development. Some of these targets include: GTP cyclohydrolase I; arginine kinase; tropomyosin; sodium pump alpha subunit; UBQLN1; calreticulin and synapse associated protein.

Creatine deficiency due to a novel nonsense mutation in the arginine:glycine amidinotransferase gene. *R. K. Bai[1], K. J. Wierenga[2], D. P. Dimmock[1], L. Y. Tang[1], L. J. C. Wong[1]* 1) Molecular & Human Genetics, Baylor College of Medicine, Houston, TX; 2) Division of Medical Genetics, University of Miami, Miami, FL.

Arginine:glycine amidinotransferase (AGAT) catalyzes the first step of creatine synthesis by combining arginine and glycine to form guanidinoacetate (GAA) and ornithine. AGAT is encoded by GATM gene, which has nine coding exons and has been mapped to 15q21.1. The cDNA is 1272 bp long encoding a protein of 423 amino acids. GAA is subsequently converted to creatine by the action of guanidinoacetate methyltransferase (GAMT). To date, only one affected family has been described with this disorder. In this kindred, two affected siblings, and subsequently their second cousin and another sibling were found to be homozygous for a nonsense mutation, c.446G>A, p.W149X, resulting in an undetectable mRNA by RT-PCR, and undetectable AGAT activity. The two female siblings, aged 4 and 6 years, had presented with mental retardation and severe brain creatine deficiency detected by NMR Spectroscopy. Cerebral deficiency was reversed by oral creatine supplementation, with favorable clinical response. Here we report a novel homozygous nonsense mutation, c.505C>T, p. R169X in two adult siblings, a 26 year old female and a 23 year old male, children of a first-cousin relationship. Both had significant developmental delay in early childhood without diagnosis, and adult-onset of proximal muscle weakness with undetectable GAA levels in plasma and urine as part of a genetic evaluation. The p.R169X mutation results in a truncated protein lacking all three important amino acid residues, C407, H303 and D254, that form the proposed catalytic triad. Evaluation of the parents revealed that each is a carrier for the p. R169X mutation. Consistent with the previously reported truncating mutation, both siblings have extremely low levels of AGAT mRNA by quantitative RT-PCR (~5% of the controls). The mothers AGAT mRNA level is ~45% of the controls. This is the second mutation found in the GATM (AGAT) gene and the second kindred described with arginine:glycine amidinotransferase (AGAT) deficiency.

***In vitro* and *in vivo* analysis of the AMP-activated protein kinase alpha subunit (AMPK) carboxyl-terminus reveals vital roles in both localization and function.** *T. Williams, N. Kazgan, LJ. Forsberg, JE. Brenman*
Neuroscience, UNC CHAPEL HILL, Chapel Hill, NC.

Often referred to as an energy sensor, AMP-activated protein kinase (AMPK) is a serine-theronine kinase whose function has been implicated in cell polarity and cell division, in addition to energy metabolism. AMPK is a heterotrimer composed of a catalytic subunit and two regulatory subunits, and . There are regions within each subunit that have considerable conservation across species. Specifically, the amino acid sequence of the kinase domain within AMPK is highly conserved. However, the carboxyl-terminus lacks conservation with the exception of the last 20 amino acids. We hypothesize that the last 20 amino acids are vital to the structure, function, and/or localization of AMPK. To test this hypothesis we generated mammalian constructs, GFP tagged full length AMPK (pGFP-AMPKwt) and Truncated AMPK (pGFP-AMPKtrunc) missing the last 20 amino acids, and performed transient transfection in HeLa cells. *In vivo*, we utilized transgenic Drosophila fruit flies expressing either full length AMPK or the truncated version. Both *in vitro* and *in vivo* experiments demonstrated AMPK localization in both the cytoplasm and nucleus, with the truncated version enriched in the nucleus. HeLa cells transfected with pGFP-AMPKwt and pGFP-AMPK-trunc were treated with leptomycin B (LMB) in order to determine if the observed nuclear localization was due to a block in CRM1 mediated nuclear export. Post LMB treatment pGFP-AMPKwt expression localized to the nucleus suggesting AMPK nuclear export is CRM1 mediated. In addition, the last 20 amino acids appear to be vital for AMPK function *in vivo*. AMPK null flies are 2nd instar larval lethal and have neuronal phenotypes. Furthermore, while wildtype AMPK is able to rescue both the neuronal phenotypes and lethality, the truncated transgenic flies lacking the final 20 amino acids are unable to rescue any of the AMPK null phenotypes in flies. In conclusion, our studies indicate that the last 20 amino acids of AMPK are important for localization and function. We intend to investigate whether the truncated function is altered, reduced, or completely diminished.

BAC-FISH assay for FGFR1 rearrangement in 8p12-MPD and report of a new patient with t(8;9)(p12;q33). *R. Fraser[1], M. Nicola[1], J. Suttle[1], N. Patton[2], S. Moore[1]* 1) Division of Molecular Pathology, Institute of Medical and Veterinary Science, Adelaide, Australia; 2) Division of Haematology, Institute of Medical and Veterinary Science, Adelaide, Australia.

Fibroblast Growth Factor Receptor 1 (FGFR1) gene rearrangement is the molecular hallmark of a recently recognised group of myeloproliferative disorders (MPD) that affect the hemopoietic stem cell. This disease mimics CML in that it has an early chronic phase and a more aggressive blastic phase, which is usually fatal within 12 months of diagnosis. A new small molecule tyrosine kinase inhibitor, PKC412, has been demonstrated to inhibit the proliferation of FGFR1-rearranged cells and clinical trials are underway to determine its therapeutic efficacy. Since FGFR1 is a promiscuous gene, with rearrangement at 8 different chromosomal loci reported so far, detection of FGFR1 rearrangement by BAC-FISH is of obvious use in the identification of patients who may benefit from treatment with PKC412. A 63 year old woman presented to our Unit in July 2007 with MPD showing marked bone marrow eosinophilia and the cytogenetic finding of t(8;9)(p12;q33). Utilising BACs from the Childrens Hospital Oakland Research Institute we demonstrated that the FGFR1 gene was disrupted in this patients malignant cells and that she was therefore a candidate for treatment with PKC412. She commenced therapy in January 2008 and showed a reduction in white cell count. Dose escalation resulted in further improvement, although her response has now plateaued. We have used BAC-FISH as a rapid and sensitive method to provide an individualised FISH result within 48 hours. Since new targeted therapies are under constant development, fast confirmation of chromosomal breakpoints and resultant gene disruption is required to identify those patients who may obtain clinical benefit from these novel therapies. This technique also allows monitoring each patients responses to therapy should their tumor burden fall below the level detectable by classical cytogenetic evaluation.

Recurrent 1q21.1 microdeletions associated with variable disease phenotypes. *H. Mefford[1], A. Sharp[2], B. Conrad[3], T. Walsh[4], S. Antonarakis[2], C. Chen[5], G. Gimelli[6], S. Schwartz[7], J. Sutcliffe[8], S. Knight[9], J. Sebat[10], C. Romano[11], C. Schwartz[12], J. Veltman[13], B. de Vries[13], J. Vermeesch[14], J. Barber[15], L. Willatt[16], M. Tassabehji[17], E. Eichler[1,18], The 1q21.1 Deletion Consortium* 1) Genome Sciences, Univ of Washington, Seattle, WA; 2) Genetic Medicine & Development, Univ of Geneva, Switzerland; 3) Human Genetics, Bern Univ Children's Hospital, Switzerland; 4) Medicine, Univ of Washington, Seattle, WA; 5) Applied Biosystems, Foster City, CA; 6) Instituto G. Gaslini, Genova, Italy; 7) Univ of Chicago, Chicago, IL; 8) Vanderbilt Univ, Nashville, TN; 9) Wellcome Trust Centre for Human Genetics, Oxford, UK; 10) CSHL, Cold Spring Harbor, NY; 11) IRCCS Assoc. Oasi Maria Santissima, Troina, Italy; 12) Greenwood Genetic Center, Greenwood, SC; 13) Radboud Univ Nijmegen Medical Centre, The Netherlands; 14) Human Genetics, Catholic Univ of Leuven, Belgium; 15) Salisbury District Hospital, Salisbury, UK; 16) Medical Genetics, Univ of Cambridge, UK; 17) Medical Genetics, Univ of Manchester, UK; 18) HHMI, Seattle, WA.

We present a series of patients with recurrent reciprocal rearrangements of chromosome 1q21.1 associated with a variable spectrum of phenotypes. We identified 20 individuals with a recurrent 1.35-Mb deletion from a screen of 5243 patients with mental retardation and/or congenital anomalies. The deletions are *de novo* in five cases, inherited from an apparently unaffected parent in seven cases, and of unknown inheritance in eight cases. The deletion was absent in 4737 controls, representing a significant association with disease ($p<3x10^{-6}$). Considerable variability in expressivity was observed among probands including mental retardation, microcephaly, cardiac abnormalities and cataracts. The reciprocal duplication seems enriched in affected individuals but too few cases (8/5243) were observed to conclude statistical significance. We predict that screens for structural variants across large patient pools with diverse and complex phenotypes will reveal other recurrent molecular lesions that elude syndromic classification. Clinical recognition of these patients may be most effective by diagnosis based on genotype rather than phenotype.

FGFR2 SNPs are associated with contralateral breast cancer in the WECARE Study. *S. Teraoka[1], A. Reiner[2], J. L. Bernstein[2], R. W. Haile[3], P. Concannon[1], The WECARE Study Consortium* 1) University of Virginia, Charlottesville, VA; 2) Memorial Sloan Kettering Cancer Center, NY, NY; 3) University of Southern California, LA, CA.

Recent genome-wide association studies have identified single nucleotide polymorphisms (SNPs) in the *FGFR2* gene that are associated with breast cancer risk. To assess the role of variation at *FGFR2* in risk of developing second primary breast cancer, we genotyped 6 SNPs in *FGFR2* intron 2 in all 2105 subjects in the WECARE Study. The WECARE Study is a multi-center, population-based, case-control study designed to examine gene-environment interactions and risk of contralateral breast cancer (CBC). The study population included 708 women with CBC (cases), and 1397 women with unilateral breast cancer (UBC) (matched controls), all of whom were ascertained through five population-based cancer registries in the U.S. and in Denmark. Controls were matched 2:1 on birth year, year of breast cancer diagnosis, registry, race, and counter-matched on registry-reported radiation exposure. Minor alleles at three of the *FGFR2* SNPs were associated with CBC; rs2981582 (rate ratio (RR) = 1.3, 95% confidence interval (CI) = 1.1-1.7), rs2981578 (RR = 1.4, 95% CI = 1.1-1.8), and rs1078806 (RR = 1.3, 95% CI = 1.0-1.6). No significant interactions were observed for any of these SNPs with either radiation dose or age at onset. A consideration of haplotypes formed from these SNPs suggests that the association between *FGFR2* and CBC is haplotype-based. These findings further support a role for variation at the *FGFR2* locus in modifying risk for breast cancer.

PAX3 and MITF oppositely regulate a melanogenesis nodal point in metastatic melanoma cell lines. *M. Eccles[1], S. He[1], A. Jeffs[1], C. Li[1], A. Glover[1], J. Ineson[1], E. Marshall[2], H.-S. Yoon[1], B. Baguley[2]* 1) Dept of Pathology, Dunedin School of Medicine, University of Otago, Dunedin, New Zealand; 2) Auckland Cancer Society Research Centre, The University of Auckland, Auckland, New Zealand.

PAX3 and MITF are markers of the neural crest cell lineage. These two proteins form part of a network of proteins that regulate neural crest and melanocyte developmental pathways. We are interested in whether PAX3 and MITF proteins control a gene regulatory nodal point in melanoma cells, just as in melanocytes, and whether this has gone awry in melanomas, thereby providing a mechanism for failure of proper differentiation in melanoma onset. In this research we found that PAX3 and MITF were expressed in skin melanocytes, nevi, and primary and metastatic melanomas, but they were not necessarily expressed together. That developmental pathways could be part of subsequent progressive oncogenic changes is suggested, as there was a progressive acquisition of PAX3, or MITF and co-expression of the proliferation marker Ki67 with increasing malignancy in melanoma. Using a panel of metastatic melanoma cell lines, we found that PAX3 and MITF were relatively infrequently expressed together, and in knock down experiments in melanoma cells, neither *PAX3* nor *MITF* were dependent on the other genes expression. Melanogenesis (synthesis of melanin pigment) typifies melanocyte differentiation and is present in most melanomas. In representative melanoma cell lines, we found that the knockdown of *PAX3* or *MITF* led to reciprocal effects on melanogenesis and other morphological features, without induction of cell death. However, in other melanoma cell lines, *PAX3* or *MITF* knockdown did lead to significant apoptotic cell death, and concomitant decrease in *BCL-2* and/or *CDK2*expression. Taken together, our data suggest that the integrity of the melanogenic pathway and its regulation is an important feature in the proliferation and survival of melanoma cells. We also show that the *PAX3/MITF* gene regulatory nodal point is present in melanoma, but appears to malfunction in terminal differentiation. A proportion of melanoma cell lines were not dependent on melanogenic pathways or regulation for their proliferation and survival.

Utility of 3D Imaging for Genetic Counseling and Perinatal Planning for Maxillonasal Dysplasia: In Utero Natural History of Binder Syndrome Phenotype. *J. Wente[1], R. F. Hume, Jr.[3], K. Norris[2, 3], L. Zak[4], M. Estes[3], S. Kerger[3], T. Kuperberg[3], D. C. Willis[3], L. S. Martin[5]* 1) Family and Child Sciences Prog, Florida State University, Tallahassee, FL; 2) Diagnostic Medical Sonographer Program, Tallahassee Community College; Tallahassee, FL; 3) Center for Maternal Fetal Medicine; Tallahassee Memorial Healthcare; Tallahassee, FL; 4) Neonatal Intensive Care Unit, Tallahassee Memorial Healthcare, Tallahassee, FL; 5) Nemours Children's Clinic; Division of Genetics; Jacksonville, FL.

Case Report: 25 yo G2 P1001, routine obstetrical sonography at 18 weeks failed to visualize fetal nose. TARGET US identified severe midfacial hypoplasia and absent nasal bone. Differential diagnosis includes lobar holoprosencephaly syndromes and aneuploidy. After genetic counseling and repeat fetal imaging (3D Facial Mask), family chose amnio; 46 XY. Fetal echocardiography and subsequent serial fetal imaging confirmed isolated midfacial defect consistent with Maxillonasal Dysplasia (Binder). Perinatal genetic counseling was enhanced by the perceived clarity of 3D facial imaging. Serial multidimensional fetal sonography from 18 through 37 weeks gestational age provides the earliest 3D images of the in utero natural history of maxillonasal dysplasia which significantly facilitated family understanding of issues and risks for their son, even with apparently normal chromosomes. Continued fetal care addressed evolving phenotype: no evidence of holoprosencephaly, normal fetal echocardiography, transient polyhydramnios, and progressive but hypoplastic growth of nose. Choanal atresia could not be excluded. Neonatal management plans were facilitated by clarity of fetal images. Cesarean section at 37 weeks due to Intrauterine growth failure (IUGR), 2 kg 5%tile SGA, APGAR 8/8, choanal atresia was excluded, neonatal imaging confirmed isolated maxillonasal dysplasia, and neonatal course was uncomplicated. Conclusion: Our case provides further evidence for the benefit of advanced imaging for prenatal diagnosis and the enhanced understanding of fetal condition, the Binder Phenotype. More important for our patient was her better understanding of fetal disorder due to 3D findings.

Development of Genome Wide Association Database in Japanese Integrated Database Project. *A. Koike[1], N. Nishida[2], I. Inoue[3], S. Tsuji[4], K. Tokunaga[2]* 1) Cent. Res. Lab., Hitachi, Ltd., Japan; 2) Graduate School of Medicine, University of Tokyo; 3) Tokai University School of Medicine; 4) Department of Neurology, Graduate School of Medicine, University of Tokyo.

The recent progress in the large-scale and high-density genome wide association studies (GWAS) has brought us valuable clues to elucidate genetic factors associated with various complex diseases. The management of vast amounts of data and analysis results produced by GWAS projects and schematics of their data sharing among researchers are becoming concerns. In NCBI, the dbGAP has been launched in 2006 as a GWAS repository system, while in EBI, the EGA has been created in 2007 as a repository system for phenotype-genotype relationships. Our organizations (Univ. of Tokyo, Univ. of Tokyo Hospital, Univ. of Tokai, and Hitachi, Ltd.) involved in the integrated database project supported by Ministry of Education, Culture, Sports, Science and Technology (MEXT) has created GWAS database (https://gwas.lifesciencedb.jp/) to achieve continuous and intensive management of GWAS data and data sharing among researchers, and widely call for data-deposit of GWAS data. In the GWAS database, information on the study design, quality control protocols, allelic and genotypic frequencies, and statistical results are stored as freely accessible data, while individual genotyping data and raw data are stored as restricted data and cannot be accessed without special permission. The graphic viewer to display statistical results along with other information such as copy number variations and exon information has also been provided to facilitate identification of disease-related SNPs. Furthermore, comparisons among various study results produced by different institutions and different platforms, and meta-analysis for these results are available. The database is designed to be friendly for researchers not familiar with GWAS to promote disease related studies. In this presentation, we introduce the overview of database structure and methods for SNP quality control and for statistical genetic analysis and data management policies. Acknowledgements: This study was supported by the integrated database project of MEXT.

Evaluation of Imputation-based Association in and around Integrin--M (ITGAM) gene and Replication of Robust Association between a Non-synonymous Functional variant in ITGAM and Systemic lupus erythematosus (SLE). *SK. Nath[1], X. Kim-Howard[1], H. Deshmukh[1], S. Han[1], P. Viswanathan[1], J. Guthridge[1], P. Guffney[1], K. Moser[1], R. Kimberly[1], K. Kaufman[1], C. Jacob[1], J. James[1], S. Bae[4], J. Anaya[3], K. Matsuda[2], M. Alarcon-Riquelme[6], T. Vyse[5], J. Harley[1]* 1) USA; 2) Japan; 3) Colombia; 4) Korea; 5) UK; 6) Sweden.

Recently, we identified (Nat Genet. 40:152-154, 2008) a novel non-synonymous variant (R77H substitution), rs1143679, at exon-3 of ITGAM associated with SLE. The objectives of the present study are to (a) assess whether single or multiple causal variants from the same gene or any nearby gene(s) are involved in SLE susceptibility using an imputation-based association test, (b) assess the robustness of the genetic association across 9 ethnically diverged samples (~12000), including European-American (EA), Hispanics (HI), Korean, Japanese, United kingdom (UK), Colombian and Mexican, and (c) resequence exon-3 to identify novel variants and reassess the causal association. Our imputation-based association analysis confirms the ITGAM is the major susceptibility gene for SLE (log 10 Bayes factor = 20 for European-American). The association between rs1143679 and SLE is reconfirmed and replicated in 5 case-control samples including EA ($P = 1.0 \times 10^{-8}$, OR = 1.7, 95% CI = 1.4-2.1), HA ($P = 2.8 \times 10^{-5}$, OR = 2.1,CI = 1.4-2.9), UK ($P = 3.0 \times 10^{-6}$, OR = 2.1,CI = 1.5-2.8), Mexican (P = 0.002, OR = 1.6, CI = 1.2-2.3) and Colombian ($P = 3.6 \times 10^{-7}$, OR = 2.2, CI = 1.6-3.1), and 2 trios including UK (P = 0.003, OR = 1.6, CI = 1.2-2.4) and Mexican (P = 0.01, OR = 1.7, CI = 1.2-2.6). A meta-analysis combing all data greatly reinforces the association ($P = 1.7 \times 10^{-45}$, OR = 1.8, CI = 1.6-1.9). However, ITGAM association is not observed in the Korean and Japanese samples, in which the rs1143679 is monomorphic (risk allele A). Finally, resequencing exon-3 did not identify any novel variant. Therefore, taken together with our earlier findings, our results demonstrate the $_{M2}$-integrin adhesion pathway in disease development, especially in the European- and African-derived populations, but probably not in Asian derived populations.

Severe Neonatal Complications in an Infant Girl with Jansen's Metaphyseal Chondrodysplasia. *C. Kozma[1], N. Scribanu[1], K. Abubakar[1], R. Lachman[2]* 1) Dept Pediatrics, Georgetown Univ Hosp, Washington, DC; 2) Cedars-Sinai Medical Center, Los Angeles.

Jansens metaphyseal chondrodysplasia is a severe rare form of short-limbed dwarfism. It is caused by mutations in the PTH/PTHrP receptor gene. The disorder is inherited in an autosomal dominant fashion. Case reports describing neonatal complications associated with the syndrome are very few. We report an affected girl who experienced respiratory distress, severe feeding difficulties, and significant hypotonia as well as motor deficits. She was the product of the second pregnancy to 27-year-old Salvadoran woman whose pregnancy was complicated by polyhydramnios and short femur. The family history was unremarkable. Genetic amniocentesis done at 32 weeks of gestation revealed normal karyotype. The baby was born vaginally at 36 weeks of gestation. Her head circumference was 32.5 cm (60th centile); weight 2903 gms (60th centile), length 43 cm (3rd -10th centile). Physical examination showed flat supra orbital ridge, small mouth, small thorax, moderate pectus carinatum, and a slight bowing of the legs. The nails and fingers were normal. She required brief mechanical ventilation and oxygen treatment for a few days. Due to her short stature, X rays were done revealing the long bones to be proportionately short and to have a moth-eaten appearance with diffuse osteoporosis. There was bilateral hip dislocation. The proximal ends of the humeri as well as the distal ends of the ribs had a severe flared and raggy appearance. The ribs appeared shortened. The metaphyseal ends of the major long bones showed irregularity, slight cupping and slight splaying of the metaphyses in addition to a celery stalked appearance. The talus and calcaneus demonstrated focal areas of bone sclerosis and osteoporosis as well as popcorn density. Throughout her stay, she had significant generalized hypotonia, absent suck and very decreased neonatal reflexes. Feeding was done by gavage. Her calcium and phosphorus level were normal. She died at the age of 5 weeks of wide spread necrotizing enterocolitis. This case adds to the further documentation of the natural history of this disorder.

A New de novo Balanced Translocation Breakpoint Truncating the Autism Susceptibility Candidate 2 (AUTS2) Gene in an Autistic Patient. *X. Huang[1], Y. S. Zou[1,2], T. A. Maher1[1], S. Newton[1], J. M. Milunsky[1,2,3]* 1) Dept Human Gen, Boston Univ Sch Med, Boston, MA; 2) Dept Pediatrics, Boston Univ Sch Med, Boston, MA; 3) Dept Genet and Genom, Boston Univ Sch Med, Boston, MA.

Autism Spectrum Disorder (ASD) is a developmental disorder of the central nervous system of largely unknown etiology. Truncated AUTS2 at 7q11.2 by a translocation breakpoint has been reported in four unrelated ASD/mentally disabled patients. Here we present a new patient carrying a new de novo balanced translocation that truncates the AUTS2 gene. He is a mildly dysmorphic 5-year old boy with delays in language, finemotor, and visual receptive skills who has been diagnosed with pervasive developmental disorder - not otherwise specified (PDD-NOS). His score on the childhood autism rating scale (30.5) indicates mild-moderate autism. Chromosome analysis revealed a translocation: t(6;7)(q14;q11.2)dn. Affymetrix 500K SNP array analysis was normal, thus supporting a balanced reciprocal translocation. Simultaneous molecular genetic testing of Fragile X syndrome, as well as NLGN3 and NLGN4 were normal. BAC-FISH mapping revealed that the chromosome7 breakpoint is within intron 1 of AUTS2. The breakpoint of 6q14.1 is between RP11-352K16 and RP11-213K12, where there are abundant retrotransposon long interspersed element-1 (LINE-1) elements and no reported genes. The mild-moderate autism seen in our patient with truncated AUTS2 gene is consistent (probably milder) with previous reports. It indicates that the AUTS2 gene, originally identified as KIAA0442 from a library of large brain-expressed transcripts previously linked to autism and mental retardation, plays a critical role in the etiology of ASD.

Characterization of the immune response to Helper Dependent Adenovirus for Gene Therapy of Hemophilia A.

M. Suzuki[1], V. Cerullo[1], N. Bruetti-Pierri[1], M. Seiler[1], V. Mane[1], C. Clarke[1], R. Cela[1], L. Medima-Kauwe[3], B. Lee[1,2] 1) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Howard Hughes Medical Institute, Houston Tx; 3) Cedars-Sinai Medical Center, University of California Los Angeles, Los Angeles CA.

Hemophilia A is a common inherited coagulopathy caused by deficiency of factor VIII (FVIII) activity. Previously, we focused on gene therapy of hemophilia A using helper dependent adenovirus (HDAd) encoding B-domain deleted human FVIII in both dog and mouse models. However, host antibody response to hFVIII has been a limitation of these therapies. We hypothesized that the innate immune response to HDAd may correlate with the induction of adaptive immune response to transgene product delivered with these vectors. In the present study, we investigated the cellular and molecular mechanisms related to induction of the innate immune to infection with HDAd using genetically modified mice deficient in components of this system. Toll-like receptors (TLRs) are innate receptors that sense microbial products. We first asked whether the class of TLRs is involved in the immune response following the intravenous injection of HDAd. To test this, we injected the HDAd encoding LacZ transgene into Myd88 knock-out (Myd88-KO) mice. Myd88 is a well characterized key signal mediator of TLRs signals. We measured cytokine and chemokine production in serum of mice injected with HDAd at early time points. Myd88-KO mice showed significant reduction of proinflammation molecules compared with WT C57Bl/6 mice after HDAd treatment. Similarly, surface TLR2 and endosomal TLR9-KO mice injected with HDAd exhibited significant reduction of expression of these cytokines albeit less so then that observed in Myd88-KO mice. These results indicated that systemic administration of HDAd activates the immunity through TLR-dependent pathways leading to the induction of innate immune response. Myd88-KO mice injected with HDAd exhibited high and long-term expression of transgenes compared with WT mice. By modulating signaling via these receptors we may be able to decrease the innate and adaptive immune response to HDAd and/or transgene products.

Functional characteristics of a 4.5kb non-coding DNA found to be associated with acute graft-verse-host disease.

J. Li[1], B. Zhang[1], X. Zhang[1], L. Tabellini[1], W. Fan[1], P. Martin[1], J. Chien[1,2], H. Warren[1], B. Storer[1], L. Zhao[1,2], J. Hansen[1,2] 1) Fred Hutchinson Cancer Research Center, Seattle, WA; 2) University of Washington, Seattle, WA.

Acute graft-versus-host disease (aGvHD) following allogeneic hematopoietic cell transplantation (HCT) is the major cause of morbidity and mortality. The genetic determinants modulating the incidence and intensity of aGvHD are largely unknown. Recently, we have genotyped 1425 HCT recipients and 1506 donors using Affymetrix 5.0 GeneChip. A total of 130 copy number polymorphisms (CNPs) were identified in our study population. A CNP association analysis with aGvHD revealed one signal consisting of 4.5 KB deletion mapping to an intergenic region on chromosome 8. This CNP was initially identified by Hinds *et al.* (Nat Genet. 2006, 38:82-5) and independently validated by Kidd *et al.*(Nature. 2008, 453:56-64). We performed a detailed sequence analysis of this CNP region using the consensus sequence from the Human Genome. Three well-conserved regions were identified which contain several known motifs including TATA box (TATAAA), and a 300bp down-stream sequence harboring part of an ORF sequence which overlaps with sequences encoding components of ten unique zinc finger proteins as well as several other transcriptional/translation regulators. Several ESTs , a zinc finger transcription factor, a eukaryotic translation initiation factor and a zinc transporter locate immediately downstream of the identified CNP. Preliminary results from gene expression profiles of aGvHD patients indicate the involvement of a group of genes encoding zinc finger proteins and transcriptional/translation regulators as seen in our CNP sequence analysis, supporting our hypothesis of a novel functional component in this identified non-coding region on chromosome 8. Further validation of this association is planned. Besides gaining insight into the biology in acute GvHD, we believe this analysis, coupled with the experimental validation strategy would be useful to study other genetic discoveries, which locate within gene desert regions. * Corresponding authors: jhansen@fhcrc.org and lzhao@fhcrc.org.

Complex epigenetic abnormalities are not detected by targeted methylation analysis. *S. Horike[1,7], M. Meguro-Horike[1,7], J. C. Ferreira[1], A. C. Smith[1,4], C. Shuman[1,2,3], W. Meschino[5], S. W. Scherer[1], E. Zackai[6], R. Weksberg[1,2,3,4]*
1) Program in Genetics and Genomic Biology, The Hospital for Sick Children, Toronto, Canada; 2) Division of Clinical and Metabolic Genetics, The Hospital for Sick Children, Toronto, Canada; 3) Department of Molecular and Medical Genetics, University of Toronto, Toronto, Canada; 4) Institute of Medical Sciences, University of Toronto, Toronto, Canada; 5) Division of Clinical and Metabolic Genetics, North York General Hospital, Toronto, Canada; 6) Division of Human Genetics and Molecular Biology, Childrens Hospital of Philadelphia; 7) Frontier Science Organization, Kanazawa University, Kanazawa, Ishikawa, Japan.

Over a 10 year period blood samples from 63 individuals with growth restriction and RSS-like features were collected. Our goal was to identify causal epigenetic abnormalities e.g. UPD 7 (mat), methylation changes at 11p15, and new epigenetic alterations. We evaluated the methylation status of 6 imprinted loci, on chromosomes 7, 11, 14 & 15 including ICR1, ICR2, *H19* promoter (at 11p15), *PEG1/MEST*, *MEG3/GTL2*, *SNRPN* CpG islands. We previously reported UPD 7 (mat) in 3 cases. Epigenetic alterations on chromosome 11p15.5 were identified in 10 cases. This included one set of MZ twins discordant for both IUGR & for 11p15.5 loss of methylation. In addition, in one case we detected methylation changes across all 4 chromosomes tested consistent with maternal UPD and prompting consideration of more complex diagnoses. The newborn clinical presentation for this case included birthweight 3%, head circumference 50%, hemiatrophy and hypospadias. Although this patient is phenotypically male, we did not detect any Y-specific PCR product. Follow-up clinical information included karyotype: 46, XY (and 1/100 69, XXY). No further blood/tissue samples could be obtained. We present this case to demonstrate that complex epigenetic abnormalities will not be detected if testing is limited to single targeted assays.

Congenital aural atresia type I, report 3 Mexican patients. *L. Hernandez[1], G. Juarez[2], D. Gomez[3], E. Hernandez[4]* 1) AUDIOLOGIA, INSTITUTO NACIONAL DE REHABILITACION, MEXICO D.F; 2) NEUROPSICOLOGIA, INSTITUTO NACIONAL DE REHABILITACION, MEXICO D.F; 3) INSTITUTO NACIONAL DE REHABILITACION; 4) FACULTAD DE ESTUDIOS SUPERIORES IZTACALA BIOLOGIA.

Is a rare anomaly, occurring in approximately 1 in 10,000 live births. In only about 5% of cases does one find atresia without concomitant Microtia. The CAA (congenital aural atresia) phenotype varies from mild manifestations, has been divided into three forms based on increasing severity. Type I, with narrowing of the external auditory canal and hypoplasia of the tympanic membrane and middle ear cavity. Type II, to severe manifestations, including absence of the middle ear in combination with anotia. Type III, bony atresia of the external auditory canal, and hypoplasia of inner ear structures. We report 3 Mexican patients Case I: female patient 6 year old, both hearing loss, onset at 5 years old, examination physical both normal pinna , right aural atresia, left narrowing of the external auditory canal. Audiometric test showed mild conductive hearing loss. Speech audiometric test showed conductive deficit. Report computed tomography right aural atresia, left narrowing of the external auditory canal. Case II: male patient 10 year old, right hearing loss, onset at 1 years old, examination physical both normal pinna , right aural atresia. Audiometric test showed right mild conductive hearing loss. Speech audiometric test showed right conductive deficit. Left Normal audition. Report computed tomography right aural atresia. Case III: male patient 6 year old, left hearing loss, onset at 3 years old, examination physical both normal pinna , left aural atresia. Audiometric test showed left mild conductive hearing loss. Speech audiometric test showed left conductive deficit. Right Normal audition. Report computed tomography left aural atresia .

International Patient Survey of Treatment Satisfaction for Mucopolysaccharidosis I. *W. Harris[1], K. Harkins[2], B. Wedehase[3], C. Lavery[4], N. Durkin[4], G. F. Cox[1]* 1) Genzyme, Cambridge MA; 2) Canadian MPS Society, North Vancouver, BC; 3) National MPS Society, Durham, NC; 4) Society for Mucopolysaccharide Diseases, Amersham, Buckinghamshire, UK.

Background: Patient satisfaction is an important gauge of treatment success. We asked patients with mucopolysaccharidosis I (MPS I) and their parents/guardians how treatment with enzyme replacement therapy (ERT, laronidase, Aldurazyme) and/or hematopoietic stem cell transplantation (HSCT) met their expectations. Methods: With MPS societies, Genzyme designed and distributed an 8-page questionnaire between 9/06 to 11/07 in the UK, US, Brazil, Canada, Germany, Poland, Netherlands, Japan, and Australia. Results: Response rate was 45% (238 completed surveys). Gender ratio was equal; phenotype distribution was 60% Hurler, 21% Hurler-Scheie, and 18% Scheie; 25% were <6 y; 45%,6-17 y, and 29% 18 y; 43% had HSCT (years since HSCT: <3: 22%, 3-5: 27%; >5: 52%), 58% received ERT (11% short-term peri-HSCT, 20% for <3 y, 27% for >3 y) and 8% received neither. Independent of treatment expectations, quality of life was rated as excellent/very good by 81% of respondents. The lowest rated symptom was musculoskeletal, rated by 60% as fair/poor. Mental development was rated by 39% as fair/poor; 60% reported learning difficulties and 49% reported mood disorders (anxiety, stress, depression). Among HSCT patients, 60% reported complications and 26% repeat procedures; however, 84% rated HSCT performance highly favorably (mean score 8.95 on a 1-10 scale) and 75% reported some/a lot of improvement on mental development. The earlier the HSCT, the greater the satisfaction. Lowest satisfaction was with cardiac, visual, and musculoskeletal symptoms. Among ERT patients, 63% rated performance highly favorably (mean score 8.14), with best ratings for quality of life, gastrointestinal, sleep, and musculoskeletal symptoms and lowest ratings for mental development, cardiac, craniofacial, and visual symptoms. The longer patients were on ERT, the greater the satisfaction. Conclusions: MPS I patients receiving therapy give overall positive reports on physical and emotional health and high satisfaction rates for both HSCT and ERT.

Use of multiple ethnic groups to identify causal breast cancer risk variants in the *FGFR2* and *TNRC9* loci. *M. Udler[1,3], S. Ahmed[2], M. Maranian[2], K. Gregory[2], K. A. Pooley[2], J. Tyrer[2], P. D. Pharoah[2], J. P. Struewing[3], R. Luben[1], C. A. Haiman[4], A. Wu[4], H. Anton-Culver[5], C. Y. Shen[6], D. Kang[7], A. Lindblom[8], B. A. J. Ponder[9], K. Malone[10], E. A. Ostrander[3], A. M. Dunning[2], D. F. Easton[1]* 1) Dept of Public Health & Primary Care, Univ. of Cambridge, UK; 2) Dept of Oncology, Univ. of Cambridge, UK; 3) NHGRI/NIH, MD; 4) Dept of Preventive Medicine, Keck School of Medicine, USC, CA; 5) Dept of Epidemiology, Univ of California Irvine, CA; 6) Inst of Biomedical Sciences, Academia Sinica, Taiwan; 7) Seoul National Univ College of Medicine, Korea; 8) Karolinska Inst, Sweden; 9) CR-UK Cambridge Research Inst, UK; 10) Fred Hutchinson Cancer Research Ctr, WA.

Genome-wide association studies (GWAS) have identified new breast cancer (BC) loci. We utilized data from European, Asian and African American (AA) BC case-control studies to facilitate fine-mapping of the *FGFR2* and *TNRC9* gene regions. Associated LD blocks were resequenced in 45 European individuals, creating a catalogue of genetic variation (*FGFR2*: 25kb, 117 variants; *TNRC9*: 133kb, 175 variants). For each locus, SNPs highly correlated with the best tagSNP (rs2981582 in *FGFR2*, rs3803662 in *TNRC9*) were genotyped in BC cases and controls of European (n=14,000), Asian (n=7,000) and AA (n=2,500) ethnicity. Likelihood ratio tests (LRTs) were used to exclude SNPs with likelihoods 100 times worse than the best candidate. For *FGFR2*, we identified 28 candidate SNPs. Based on the European data alone, 3 SNPs could be eliminated by LRT. Using the Asian and AA data allowed exclusion of a further 17. SNPs showed effects in the same direction in all 3 populations (P-heterogeneity 0.05). For *TNRC9*, we identified 24 candidate SNPs. Two were excluded using European data alone and 9 more using the Asian data. Candidate SNPs had effects in the opposite direction in AAs compared to Europeans and Asians. Haplotype analysis suggested there were 2 risk haplotypes in AAs, only 1 of which also conferred risk in Europeans and Asians. These results illustrate the importance of using diverse ethnic groups in fine-mapping studies to 1) eliminate candidate SNPs and 2) replicate GWAS hits as SNP alleles may affect risk differently in distinct populations.

Homozygous deletions in pedigrees with autism and recent shared ancestry implicate heterogeneous loci and genes. *E. Morrow[1,2,3,4], N. M. Mukaddes[5], S. Balkhy[6], G. Gascon[6], S. Y. Yoo[1,2,4], J. N. Partlow[1,2,4], B. Barry[1,2,4], K. Markianos[1], C. A. Walsh[1,2,4], Homozygosity Mapping Collaborative for Autism (HMCA)* 1) Division of Genetics, Children's Hospital Boston, Harvard Medical School, Boston, MA; 2) Department of Neurology and Howard Hughes Medical Institute, Beth Israel Deaconess Medical Center, Boston, MA; 3) Department of Psychiatry, Massachusetts General Hospital, Boston, MA; 4) Program in Medical and Population Genetics, Broad Institute of MIT and Harvard, Cambridge, MA; 5) Department of Child Psychiatry, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey; 6) Department of Neurosciences and Pediatrics, King Faisal Specialist Hospital and Research Centre, Jeddah, Kingdom of Saudi Arabia.

To find inherited causes of autism-spectrum disorders, we studied families in which parents share ancestors, enhancing the role of inherited factors. Using high-density SNP microarrays, we conducted genome-wide, pedigree-based copy number analysis and homozygosity mapping. We mapped several loci, some containing large, inherited, homozygous deletions that are likely mutations. These deletions affected both coding and non-coding genomic elements. The largest deletions implicated genes, including PCDH10 (protocadherin 10) and a novel gene DIA1 (deleted in autism1, a.k.a c3orf58). A subset of genes showed additional putative mutations in patients with unrelated parents. Our findings highlight the utility of homozygosity mapping in heterogeneous neurodevelopmental disorders like autism. Finally, our results offer traction for studying the potential role of non-coding mutation in neurodevelopmental disease.

Result of the bioethics questionnaire survey of Japanese university students on genetic testings. *H. Numabe[1,2], S. Kosugi[1,2]* 1) Department of Medical Ethics, Kyoto University Gradiate School of Medicine, Kyoto, Japan; 2) Department of Clinical Genetics, Kyoto University Hospital, Kyoto, Japan.

We made the questionnaire survey of Japanese university students on genetic diagnoses. The survey was done during the course of Introduction to clinical medicine and Prejudice, human rights, and discrimination in 2007. The questionnaire was consist of properties of the student, considerations to spiritual matters, acceptability of an artificial abortion, the time of the beginning of human life, and considerations to various genetic testing. Total number of students was 223 and the mean age was 19.4 years. The result was as follows. 1. Genetic testing to confirm an existing clinical diagnosis: acceptable 176 (78.9%), not acceptable 13 (5.8%) 2. Predictive genetic testing for disorders in which clinical management may not affected by the test result: acceptable 165 (74.0%), not acceptable 14 (6.3%) 3. Genetic testing for carrier status: acceptable 152 (68.2%), not acceptable 21 (9.4%) 4. Prenatal genetic testing: acceptable 96 (43.0%), not acceptable 68 (30.5%) 5. Pre-implantation genetic testing: acceptable 77 (34.5%), not acceptable (35.9%) Acceptability of genetic testing correlates closely with students recognition of the time of the beginning of human life. Students who recognize the life start from the fertilization or within 15 days after fertilization are not acceptable to prenatal and pre-implantation tests. On the other hand, students who recognize the life start after 22 weeks of gestational age or after birth are acceptable to them. Acceptability of an artificial abortion has a similar tendency, but over 50% of students who recognize the life start from the fertilization or within 15 days after fertilization are acceptable to an artificial abortion.

Melanoma and Hirschsprung's Disease in a Family: Coincidence or Shared Susceptibility? *R. S. Wildin[1,2], J. Eichmeyer[2]* 1) Idaho State Genetic Services Program, Boise, ID; 2) St. Lukes Regional Medical Center, Boise, ID.

One heritable form of Hirschsprungs Disease (HSCR) is Waardenburg-Shah (Waardenburg, type IV) Syndrome. Three genes have been reported responsible: EDN3, EDNRB, and SOX10, with some evidence in all for either recessive or occasionally multigenic inheritance. An increased frequency of EDNRB non-synonymous variations has been observed in Malignant Melanoma (MM) patients (Soufir, et al., J NCI 2005) and an ENDRB mutation has been found in an individual with a CDK-4-positive melanoma (Soufir, et al., J Dermatol Sci 2007). The EDNRB gene product interacts with SOX10, plays a role in melanocyte growth and differentiation, and genetic and epigenetic variations in it have been observed in a range of cancers. We report a young boy with apparently isolated HSCR whose paternal uncle has a lock of white hair and whose father has a history of malignant melanoma at age 30. No one in the family has deafness, iris heterochromia, or dystopia canthorum. We hypothesize that this family harbors an endothelin receptor pathway mutation that predisposes to MM, pigment anomalies, and/or HSCR, with expression depending, perhaps, on the presence of other interacting predisposing genes. Testing is underway. If true, this would alter the genetic counseling and preventative management provided for both HSCR and MM in this context.

Nonpathogenic clonal chromosome abnormalities in donor cells post transplant. *J. Meck[1], A. Meloni-Ehrig[1], G. Erdag[2], P. Mowrey[1], J. Kelly[1], L. Matyakhina[1], T. Donohue[3], R. Srinivasan[3], R. Childs[3]* 1) Quest Diagnostics/Nichols Inst, Chantilly, VA; 2) Georgetown U Med Ctr, Washington, DC; 3) NHLBI/NIH, Bethesda, MD.

Although rare, chromosome abnormalities resulting in hematologic neoplasms in donor cells after allogeneic bone marrow or peripheral blood stem cell transplantation (PBSCT) have been reported. We describe 2 patients, one with chronic myeloid leukemia (CML) and the other with chronic lymphocytic leukemia (CLL), who showed persistence for years of a chromosomally abnormal clone that appeared in donor cells several months after PBSCT, but was not associated with disease in recipient or donor. Both patients underwent PBSCT from HLA-matched opposite sex siblings. Early post-transplant studies showed full engraftment with normal donor metaphases. Subsequent studies of both patients, however, revealed a population of karyotypically abnormal, but apparently balanced, donor cells that persisted at a low level for years without apparent clinical effect. The CLL patients donor is karyotypically normal. The CML patient donor studies are pending; however, since the initial study post-transplant showed a normal donor karyotype, it suggests that the abnormalities occurred subsequent to transplant. The CLL patient has been in remission for 5 1/2 yrs. The CML patient suffered relapse of CML with karyotypic evolution in recipient cells and simultaneous appearance of the chromosomally abnormal donor clone several months after PBSCT. He was treated successfully, and was healthy until his recent accidental death. The abnormal donor cell clone persisted for 7 years. Donor cell rearrangements in our patients are not recurrent in neoplasia except for an 11q23 rearrangement, shown by FISH not to involve the MLL gene, and a t(X;20) with breakpoints similar to ones reported infrequently in MDS. To our knowledge this is the first report of a persistent, complex chromosomally abnormal clone in donor cells with no known malignancy association. We speculate that the abnormal donor clone possibly arose due to host-related factors such as the microenvironment, residual effects of chemotherapy, or faulty DNA repair mechanisms.

Genetic Variants in the Lipoprotein Lipase Gene Are Confirmed to Be Associated with Liver Enzyme Levels in Hispanic Americans. *X. Guo[1], Y.-D. I. Chen[1], M. O. Goodarzi[1], B. Fang[1], A. Xiang[2], X. Su[1], Y. Liu[1], K. D. Taylor[1], T. A. Buchanan[2], L. J. Raffel[1], J. I. Rotter[1]* 1) Medical Genetics Inst, Cedars-Sinai Medical Ctr, Los Angeles, CA; 2) USC, Los Angeles, CA.

Elevated liver enzyme (LE) levels have been associated with the insulin resistance syndrome (IRS), but the common genetic basis underlying IRS and LE has not been well established. Heritability analyses indicate significant evidence for a genetic contribution to LE levels, and co-heritability analyses showed that LE levels share common genetic determinants with IRS in several studies. The lipoprotein lipase (LPL) gene has been shown to be associated with IR in two different cohorts of Hispanic Americans (HA). The fact that the LPL gene was associated with both Gamma-glutamyl transferase (GGT) and IR in HA families recruited through the Insulin Resistance Atherosclerosis Study (IRAS) Family Study has suggested LPL as a common gene underlying GGT levels and IR. We study here the role of genetic variants in the LPL gene on GGT levels using 618 non-diabetic offspring from 160 HA families ascertained through a proband with hypertension. GGT was measured by enzymatic colorimetry. Six single nucleotide polymorphisms (SNPs) known to be in the same block in the LPL gene were genotyped in these samples. The generalized transmission disequilibrium test as implemented in the QTDT program was used in the association analysis. To avoid false positives derived from population stratification, the within family variance component was used for the association testing. After adjusting for age, sex, and body mass index, significant association with GGT was found for SNP Ser447Stop/rs328 ($p = 0.019$). Haplotype analysis revealed that the SNP was located at the fourth most common haplotype (GAGGGG), which was also significantly associated with decreased GGT (28.52.6 vs 32.21.2U/L, $p=0.009$). This haplotype has been previously reported as significantly associated with IRS in HA families recruited through CAD probands (Goodarzi et al., Diabetes, 53:214-220, 2004). These results confirmed that the LPL gene is a common genetic determinant for LEs and IRS in the Hispanic American population.

Effects of CYP2D6*10 on recurrence-free survival in breast cancer patients receiving adjuvant tamoxifen therapy. *K. Kiyotani[1], H. Zembutsu[2], T. Mushiroda[1], M. Sasa[3], K. Hirata[4], M. Okazaki[5], Y. Takatsuka[6], Y. Bando[7], I. Sumitomo[3], N. Hosono[8], M. Kubo[8], Y. Nakamura[1,2]* 1) Laboratory for Pharmacogenetics, Center for Genomic Medicine, The Institute of Physical and Chemical Research, Yokohama; 2) Laboratory of Molecular Medicine, Human Genome Center, Institute of Medical Science, The University of Tokyo, Tokyo; 3) Department of Surgery, Tokushima Breast Care Clinic, Tokushima; 4) First Department of Surgery, Sapporo Medical University, School of Medicine, Sapporo; 5) Department of Surgery, Sapporo Nyusen Clinic, Sapporo; 6) Department of Surgery, Kansai Rousai Hospital, Amagasaki; 7) Department of Molecular and Environmental Pathology, Institute of Health Biosciences, The University of Tokushima Graduate School, Tokushima; 8) Laboratory for Genotyping, Center for Genomic Medicine, The Institute of Physical and Chemical Research, Yokohama, Japan.

The clinical outcomes of breast cancer patients treated with tamoxifen may be influenced by the activity of cytochrome P450 2D6 (CYP2D6) enzyme because tamoxifen is metabolized by CYP2D6 to its active forms of antiestrogenic metabolite, 4-hydroxytamoxifen and endoxifen. We investigated the predictive value of the CYP2D6*10 allele, which decreased CYP2D6 activity, for clinical outcomes of patients that received adjuvant tamoxifen monotherapy after surgical operation on breast cancer. Among 124 patients examined, those homozygous for the CYP2D6*10 alleles revealed a significantly shorter recurrence-free survival compared with those homozygous for the wild-type CYP2D6*1 alleles (P = 0.0052), or compared with CYP2D6*1/*1 + *1/*10 (P = 0.017). Cox proportional hazard analysis demonstrated that the CYP2D6 genotype and tumor size were independent factors affecting recurrence-free survival. Patients with the CYP2D6*10/*10 genotype showed a significantly shorter recurrence-free survival period (P = 0.017; adjusted hazard ratio, 6.58; 95% confidence interval, 1.41-30.77) compared to patients with CYP2D6*1/*1 after adjustment of other prognosis factors. The present study suggests that the CYP2D6 genotype should be considered when selecting adjuvant hormonal therapy for breast cancer patients.

Downregulation of sodium channel 4 subunit in Huntington Disease transgenic mice. *F. Oyama, H. Miyazaki, M. Kurosawa, M. Yamada, N. Nukina* Lab Structural Neuropathology, RIKEN Brain Science Inst, Saitama, Japan.

Sodium channel 4 (4) is a recently identified auxiliary subunit of the voltage gated-sodium channels. We identified 4 as an EST that was significantly downregulated in the striatum of Huntington Disease (HD) model mice and patients. To define the molecular mechanism underlying the reduction of 4 expression, we generated transgenic mouse line expressing Venus under the control of mouse 4 promoter in brain. We established six mice lines exhibiting Venus expression primarily in striatum, cortex or both striatum and cortex. The mouse line exhibiting predominant Venus expression in striatum was crossed with HD model mice to generated double transgenic mice. The expression of Venus transcribed from 4 promoter-Venus transgene lacking exon-intron structure and 3-UTR as well as endogenous 4 was downregulated in the striatum of double transgenic mice. These results indicate that downregulation of 4 in HD model mice is dependent on its promoter.

Intrathecal (IT) enzyme replacement therapy (ERT) for symptomatic spinal cord compression (SCC) in a MPS VI child: safety, efficacy and pitfalls. *D. Horovitz[1], M. V. R. Munoz[2], M. A. Lima[1], T. Magalhaes[1], R. Costa[2], A. Pena Costa[1], L. Jardim[2], L. E. Carelli[3], J. Llerena Jr.[1], R. Giugliani[2]* 1) Instituto Fernandes Figueira, Rio de Janeiro, Brazil; 2) Hospital de Clinicas de Porto Alegre, RS, Brazil; 3) Into, RJ, Brazil.

SCC is a known complication of MPS VI, caused by bone disease and meningeal GAG storage. We present the experience with IT treatment with recombinant human Galsulfase (rhASB) in a child with SCC. To our knowledge, this is the first MPS VI patient with such therapeutic approach. At baseline the patient, ERT naive, presented walking difficulty and neurogenic bladder. Surgery was refused by the mother. 4 IT infusions (1.5mL of rhASB dilluted on 3mL of Elliotts B solution, total volume 4.5mL) were administered monthly via lumbar puncture. Evaluation 3 months after IT 4 showed improved sensitivity, reflexes, and correction of the incontinence in urodynamic studies; walking capacity worsened. Intravenous (IV) ERT was then introduced. A 5th IT was performed 5 months after IT 4. Over the next 2 weeks he developed hypotonia, loss of voluntary movements in the limbs, neck and trunk instability. CSF analysis ruled out inflammatory reaction, infection or monoclonal bands. Lifesaving spinal surgery with cervical decompression and fixation was performed. He is regaining motor function, with better muscle tone, strength and diminished hyperreflexia; sensitivity remains preserved. Despite urodynamics and sensitivity improvements, suggesting a positive response to IT ERT, motor function worsened. We believe motor loss was due to an increased cranio-cervical junction mobility secondary to ERT (IT and IV) associated to cervical instability related to MPS VI bone disease (partially compensated before ERT due to joint restriction), leading to a physical effect compressing motor pathways. Decompressive surgery remains the gold standard for SCC in MPS. In situations of high surgical risk or less advanced bone disease, however, the IT approach must be taken into account, as therapeutic or as adjunct therapy. Even with the observed motor complications, we believe IT ERT can be safe and may be used as an alternative for MPS VI patients.

Cornelia de Lange (CdL) Syndrome - Prenatal findings and autopsy review. *K. Chong[1], D. Chitayat[1], S. Keating[2], S. Hurst[3], A. Summers[3], H. Berger[4]* 1) Prenatal Diagnosis and Medical Genetics Program, Mount Sinai Hospital, Toronto, ON, Canada; 2) Pathology and Laboratory Medicine, Mount Sinai Hospital, Toronto, ON, Canada; 3) Lakeridge Health Corporation, Toronto, ON, Canada; 4) St. Michael's Hospital, Toronto, ON, Canada.

Objective: Low PAPP-A levels (0.3 MoM) and cystic hygroma in the first trimester as well as increased nuchal fold and limb abnormalities in the second trimester can be associated with CdL syndrome.

Design: Three women presented for genetic counseling following abnormal fetal ultrasounds: one in the first trimester with cystic hygroma followed by bilateral limb anomalies at 20 weeks gestation (case 1), two in the second trimester with multiple fetal anomalies. Case 2 had low PAPP-A as well as a fetal right hand malformation and intra-abdominal cyst. At 27 weeks gestation, intrauterine growth retardation was noted. Case 3 presented with increased nuchal fold, Dandy Walker variant, congenital heart disease, echogenic kidneys, ambiguous genitalia and clenched left hand.

Results: Amniocentesis for all cases revealed normal karyotypes. Due to the multiple fetal anomalies and suspicion of CdL syndrome, all 3 pregnancies were terminated. Autopsy findings were consistent with CdL syndrome. Genetic testing for the NIPBL gene demonstrated a base pair change in the gene, resulting in a premature stop codon/splice site change and a truncated protein in all three cases.

Conclusions: Severe phenotypes of CdL syndrome may present with low PAPP-A and cystic hygroma in the first trimester of pregnancy. First trimester screening with biochemistry and nuchal translucency ultrasound should be recommended for at risk couples with family/previous history of this condition.

Treatment of 654-Thalassemia in Mice with Delivery of siRNA and Antisense RNA Plasmids. *S. Xie, J. Zhang, Z. Ren, F. Zeng, S. Huang* Shanghai Inst Med Genet , Shanghai Children's Hospital, School of Medicine, Shanghai Jiao Tong University, 24/1400 West Beijing Road, Shanghai 20040, China.

To explore the feasibility of a molecular therapy strategy for 654-thalassemia, small interfering RNA vectors (siRNA, mi 1, mi 2), homologous to -globin transcripts and antisense RNA vector which targeted the specific portion of 654 aberrant pre-mRNA were constructed, followed by delivering into 654-thalassemia mice via an intravenous injection to investigate the impact on the treatment. A significant improvement of RBC counts and Hb levels were observed in the treated mice. We also found an obvious reduction of poikilocytosis plus reticulocyte counts in periphoreal blood under treatment and decreased nucleated cells in bone marrow. Histopathological analysis showed that red pulp decreased and clear white pulp marginal zone occurred in the spleens of the treated mice, as wells as the intrasinusoidal EMH loci reduced and iron accumulation ameliorated in the livers. RT-PCR and real-time quantitative RNA analysis showed that above 50% amount of -globin mRNA was down-expressed as well as the normally spliced -globin transcripts were present after transfection of siRNA and/or antisense RNA vectors into MEL and HeLa 654 cells, suggesting that the improvement of the anemic phenotype in 654-thalassemia mice may result from the restoration of the balance in /-globin biosynthesis by RNA interference and antisence RNA treatment. The results provide a potential way for -thalassemia therapy by using RNAi and antisense RNA approaches.

Avellino Corneal Dystrophy in a Korean population. *E. J. Lee[1], H. N. Kim[1], B. J. Ha[2], S. W. Kim[2], E. K. Kim[2], H. L. Kim[1,3]* 1) Dept Biochemistry, Ewha WOmans Univ, Seoul, Korea; 2) The Institute of Vision Research, Department of Ophthalmology, Yonsei University College of Medicine, Seoul, Korea; 3) Division of Center for Genome Science, Korea National Institute of Health, Korea Centers for Disease Control, Seoul, Korea.

Avellino Corneal Dystrophy (ACD) is autosomal dominant corneal dystrophy that presents to coexistence of granular and lattice deposits. Mutation (R124H) in TGF beta induced gene human clone 3, (bigh3) on chromosome 5q31 causes autosomal dominant corneal dystrophy including ACD, is only on identified in ACD (allelic homogeneity). On the other hand, bigh3 gene is known to a candidate gene of osteoporosis and diabetes. But, this is no report that bigh3 induced systemic disease of ACD patients so far. Therefore, we observed other phenotypes of ACD patients. We analyzed epidemiologic character in 58 Korean families, including 212 ACD patients and 339 unaffected relatives of ACD patients. In this study, heights, glucose levels, insulin levels, bone density were not different from normal population. But, uncorrected visual acuity is significantly different among women over 40 years-old with avellino, as compared to the normal population. Further investigation is needed to identify the correlation between genotype and phenotypes in Avellino and normal population using whole genome-wide scan or SNPs. This work was supported by the intramural grant from the Korea National Institute of Health, Korea Center for Disease Control, Republic of Korea (Project number 4845301-260).

Molecular, Cytogenetic and Immunohistochemical Analysis of a Complete Molar Pregnancy with Isodisomic Chromosome 9 Inversion. *M. Wick[1], N. Hoppman-Chaney[2], C. Runke[2], M. Parkman[2], G. V. Velagaleti[2]* 1) Dept OB/GYN, Mayo Clinic & Foundation, Rochester MN; 2) Dept Lab Genetics, Mayo Clinic & Foundation, Rochester MN.

Hydatidiform moles (HM) arise from an abnormal fertilization event, resulting in abnormal development of fetal and trophoblastic tissue. HM are subcategorized as Partial hydatidiform moles (PHM) and complete hydatidiform moles (CHM) based on clinical features, histopathology, karyotype and malignant potential. CHM have diffuse trophoblastic hyperplasia, hydropic villi, and lack of embryonic tissue. CHM are diploid, arising from the fertilization of an empty ovum with either a haploid sperm with subsequent duplication of chromosomes, or by two haploid sperm. Risk for gestational trophoblastic neoplasia is 18-28%. Given the clinical/prognostic importance of distinguishing PHM from CHM and the role of imprinting in HM, there has been increased interest in the use of molecular and immunohistochemical characterization. Here we report characterization of a complete molar pregnancy isodisomic for chromosome 9 inversion. Parenteral chromosome analysis revealed paternal 46,XY,inv(9)(p13q13). Microsatellite analysis of six chromosome 9 markers revealed 4 informative markers, with products of conception (POC) homozygous for all four markers. Microsatellite analysis of POC tissue for seven markers on chromosomes 6,7,8,10, 14, 15 and 17 revealed homozygosity at all markers; markers on 6 and 15 were informative and also homozygous for the paternal allele. These results are consistent with paternal isodisomy as the molecular mechanism for the molar pregnancy. Additionally, immunohistochemical analysis with p57, an imprinted gene with maternal expression, was negative. These studies confirm that the complete molar pregnancy resulted from fertilization of an empty ovum with a haploid sperm followed by duplication of paternal chromosomes. Although CHM is known to result from androgenic conception, very few cases have been reported where chromosome polymorphisms indicated the pathologic basis. Our results indicate the importance of chromosome polymorphisms in the diagnosis of uniparental disomy, especially in those cases where the karyotype is normal.

Detection of the Common Chromosomal Abnormalities Using Array-based MLPA Approach. *F. Zeng, Z. Ren, J. Yan, M. Chen, Y. Huang, Y. Wu* Shanghai Inst Med Genet , Shanghai Children's Hospital, Shanghai Jiao Tong University, Shanghai, China.

Aneuploidies are the most frequent chromosome abnormalities. Currently, the diagnosis of aneuploidies usually relies on karotyping analysis, which is labor- extensive, time-consuming and inefficient. Recently, we developed array-based MLPA method. It can detect more than 100 DNA sites or copy information within two days. We previously applied array-MLPA to detect DNA deletions and duplications in DMD gene, which proved the stability and reliability of the method. The present study is committed to apply array-MLPA to detect aneuploidies in clinical patients and to assess the accuracy in diagnose of aneuploidies as compared to karotyping analysis. Probes on several key gene sites on five chromosomes (13, 18, 21, X, Y) were designed, followed by detecting the copy number of each site to check the number of the chromosomes. A total of 200 clinical samples including 188 blood cell and 12 amnionic cell DNA samples were tested. 103 were checked out to be aneuploidis except 2 mosaicisms, that were completely in accordance with karotyping analysis. The results demonstrated that the array-MLPA takes on a high rate for detecting number changes in chromosomes. As a method of gene-scanning, array-MLPA has the advantages in detecting micro-chromosome changes at submicroscopic level, and some small changes were tested in part of samples with a normal karotype. It also has a significant help for checking the accurate sites of some unknown markers. With great advantage in simple operation, high efficiency and reliability, array-MLPA has a practical value and potential in the application of molecular and prenantal diagnosis for aneuploidies.

Regulatorygenomics.org: An ENSEMBL/BIOMART environment for the analysis of regulatory regions and their involvement in complex diseases. *P. Beaulieu[1], A. Graziani[1], R. Hamon[1], T. Pastinen[3], E. Harmsen[3], D. Sinnett[1,2]* 1) Centre de recherche, CHU Sainte-Justine, Montréal, QC, Canada; 2) Departement de Pédiatrie, Université de Montréal, Montreal, QC, Canada; 3) McGill University and Genome Quebec Innovation Center, Montreal, QC, Canada.

Previous efforts to identify functional disease-causing DNA variants were essentially oriented towards the coding regions (cSNPs) of candidate genes since these variants have a direct impact on the structure (qualitative changes) and function of the affected proteins. However, abnormal expression of finely regulated genes can also lead to disequilibria (quantitative changes) in different metabolic pathways and/or biological processes. Thus, investigation of the functional impact of SNPs as well as the determination of the importance of evolutionary conservation in the regulatory regions of candidate genes should improve our knowledge of complex disease aetiologies. As part of the Gene Regulators in Disease (GRID) project, we have to integrate layers of information including gene structure, SNP content, genomic patterns (i.e. CpG islands, conserved regions) and in silico analysis together with experimental results from electrophoretic mobility shift assays (EMSA) and several types of in vitro and in vivo promoter activity assays. Here, we present a web-based environment to combine, analyse and distribute the results generated by the GRID project as well as external sources of regulatory genomics and genomic-phenotype association data. Our software takes advantage of the ENSEMBL genome browser package combined with the BIOMART data management and query system. This allows an efficient integration of data collected from the various wet-labs and in silico platforms and therefore a better functional annotation of the regulatory regions of human genes.

GAPO syndrome or Cranioectodermal dysplasia? Three affected Egyptian sibs with overlapping features. *H. H. Mostafa, M. H. Zeineldin* Molecular Biosciences, University of Kansas, lawrence, KS.

GAPO and cranioectodermal dysplasia are rare genetic syndromes with relatively few world-wide reported cases. Both syndromes affect, with other systems, ectodermal, mainly hair and teeth, and skeletal tissues. In this report we describe 2 brothers and a sister from an Egyptian family with features of both GAPO syndrome and cranioectodermal dysplasia. We also describe features that have not been reported before in either of both syndromes including structural and functional CNS and connective tissues anomalies. We suggest that both cranioectodermal dysplasia and GAPO syndrome might be variable expression of one condition.

A model system of methylation establishment and unmethylation maintenance in the PWS-imprinting center region. *T. Ohta, N. Sosonkina, D. Starenki, N. Niikawa* Res Inst, Personal Hlth, Univ Hokkaido Hlth Sci, Ishikari-gun, Japan.

The human 15q11-q13 region has an imprinting domain consisting of two imprinting control centers, ASIC and PWSIC. PWSIC functions as a maternal and a paternal mark for the maintenance of the methylated and the unmethylated regions, respectively. This maternal methylation is supposed to be established in the maternal oocyte or at an early embryonic stage with a cis-factor from ASIC located at the upstream region of PWSIC. The paternal unmethylation and maternal methylation at PWSIC are maintained stably in all tissues through developmental stages and also in cultured cells. To confirm the stable maintenance, we generated several transgene (Tg) constracts including human genomic DNA of PWSIC to transfect and integrate into mouse ES cells with a positive selection marker. All positive mixed cell clones were harvested at 1 week, and at 4 weeks after one-week selection with subculture treatments in undifferentiation medium. Consequently, no new methylation at PWSIC was observed in any one-week cultured cells. In 4-week cultured cells, the control Tg constract [ACTB promoter-exon 1-SV40 polyA (PA)] showed that the CpG island of the ACTB promoter is partially methylated, while neither of two Tg constracts, PWSIC-SV40PA and ASIC-PWSIC-SV40PA, showed methylation at PWSIC. In contrast, PWSIC was highly methylated in cells with three Tg constracts, SV40 promoter-intron-PWSIC-SV40PA, SV40 promoter-intron-ASIC-PWSIC-SV40PA, and SV40PA-PWSIC-SN40PA. These results indicate that PWSIC is easily methylated with an alternative SV40 promoter, but suggest that PWSIC has its own factor to prevent new methylation as seen in chromatin boundaries.

Fast and accurate genotype imputation and trio phasing: new methods and applications to genome-wide association studies. *B. Browning, S. Browning* Department of Statistics, The University of Auckland, Auckland, New Zealand.

There is widespread interest in imputing genotypes for markers that are not genotyped in a sample of interest, but that are genotyped in a reference panel. Early applications of imputation have used a reference panel of phased haplotypes from the HapMap phase 2. The HapMap is an extremely valuable resource, but it is limited in both markers and samples. Copy number variants and many SNPs are not in the HapMap, and accuracy of genotype imputation is limited by the HapMaps small sample size.

We have developed new methods for phasing parent-offspring trios and for imputing genotypes. These methods enable imputation of genotypes at additional markers and the use of large reference panels with thousands of trios or unrelated individuals. Our methods can be used to:

1) Impute variants that are not in the phased HapMap data, but that have been genotyped on HapMap samples using high density arrays.

2) Impute variants by using trio data in a reference panel. Use of trio data directly, instead of phased haplotypes derived from trios, provides a way to account for haplotype uncertainty in the reference panel.

3) Combine data across studies that have used different high density arrays by using imputation with a large reference panel that has been genotyped on each array.

4) Phase and impute genotypes simultaneously using a mixture of unrelated individuals, parent-offspring trios, and phase-known haplotypes.

We have phased >2.5 million markers genotyped on 30 HapMap trios in <5 hours. Our trio phasing method is several orders of magnitude faster than the gold standard PHASE method, yet the proportion of heterozygous genotypes phased differently between the two methods is only 0.0081 (CEU) and 0.0038 (YRI).

Our new methods are implemented in Beagle 3.0, which is freely available from www.stat.auckland.ac.nz/~browning/beagle/beagle.html.

A Powerful Gene-Based Association Test using Optimally Weighted Markers. *M. Li[1], K. Wang[2], S. F. A. Grant[3], H. Harkonarson[3], C. Li[4]* 1) Department of Biostatistics and Epidemiology, Univ Pennsylvania; 2) Department of Genetics, Univ Pennsylvania; 3) Center for Applied Genomics, Children's Hospital of Philadelphia; 4) Department of Biostatistics, Vanderbilt University.

Large-scale candidate-gene and genome-wide association (GWA) studies genotype multiple SNPs within or surrounding a gene, including both tag and functional SNPs. The immense amount of data generated in these studies poses new challenges to analysis. One particularly challenging yet important question is how to best use all genetic information to test whether a gene or a region is associated with the trait of interest. We propose a powerful gene-based Association Test using Optimally weighted Markers (ATOM) within a gene or a region. Due to variation in linkage disequilibrium, different markers often associate with the trait at different levels. To appropriately apportion their contributions, we assign a weight to each marker that is proportional to the amount of information it captures about the trait locus. We analytically derive the optimal weights for both quantitative and binary traits, and describe a procedure for estimating the weights from a reference data set such as the HapMap. Compared to existing approaches, our method has several distinct advantages, including 1) the ability to borrow information from external data set to increase power, 2) the theoretical derivation of optimal marker weights, and 3) the scalability to simultaneous analysis of all SNPs in candidate genes/pathways. Through extensive simulations and analysis of the FTO gene in our ongoing GWA study on childhood obesity, we demonstrate that ATOM is robust and consistently increases the power to detect genetic association as compared to several commonly used multipoint association tests. The power improvement is even more pronounced when allelic heterogeneity exists. With the wide availability of genotyping arrays with increasing marker density, our method will provide a powerful approach to identifying disease loci from multiple genotyped markers.

Robust Multifactor Dimensionality Reduction Method for Detecting Gene-Gene Interaction in Bladder Cancer.

J. Gui[1], *J. H. Moore*[1,2,3], *A. S. Andrew*[1] 1) Community & Family Medicine, Dartmouth Medical Sch, Hanover, NH; 2) Dept. of Genetics, Dartmouth Medical Sch, Hanover, NH; 3) Norris-Cotton Cancer Center, Dartmouth Medical Sch, Lebanon, NH.

The central goal of human genetics is to identify and characterize susceptible genes for common complex human diseases. In 2001, Ritchie, et al proposed the Multifactor Dimensionality Reduction (MDR) method in American Journal of Human Genetics that successfully reduced the high dimensionality caused by combining multi-locus genotypes and provided a key step to facilitate detection of important gene-gene and gene-environment interactions. However, MDR classifies the combination of multi-locus genotypes into high-risk and low-risk groups based on a simple comparison of the ratios of the number of cases and controls to that in the entire data. This may cause false-positive findings when the two ratios are very close to each other. To tackle this problem, we propose the Robust Multifactor Dimensionality Reduction (RMDR) method that performs Fishers Exact Test on the case-control ratio in all possible genotype combinations. We classify the combination in the high or low risk group when the test is significant; otherwise we classify it to the unknown group. In this way, only genotypes with significant case-control ratios are considered. We expect that this approach will increase the power when heritability is low. In the simulation study, with 400 samples and a heritability of 0.025, we show that RMDR has 70% power to detect the true interaction as compared to 50% from MDR. We then apply the RMDR method to detect interactions in genotype data from a population-based study of bladder cancer.

Whole Genome Mapping Identifies Multiple Quantitative Trait Loci (QTL) for Novel Cardiovascular Disease Biomarkers. *S. Shah[1, 2], H. Chen[1], D. Thompson[1], S. Nelson[2], C. Haynes[2], J. Johnson[2], T. Stabler[1], Z. Dowdy[1], E. Hauser[2], S. Gregory[2], V. Kraus[1], W. Kraus[1]* 1) Dept Medicine, Duke Univ Med Ctr, Durham, NC; 2) Center for Human Genetics, Duke University Med Ctr, Durham, NC.

Background. Cardiovascular disease (CVD) has a strong genetic component and is heritable. We have shown that novel CVD biomarkers are also heritable. We hypothesized that genome mapping would identify quantitative trait loci (QTL) underlying these traits. **Methods.** Samples and clinical data were obtained on 365 members from a large, multi-generational, multiethnic family (N=3000). Commercial assays were used to measure paraoxonase, D-dimer, C-reactive protein (hsCRP) and glycated serum protein (GSP) from frozen serum. Genotyping was done using the Illumina linkage chip with ~5700 SNPs. Variance components implemented in SOLAR was used to calculate linkage odds (LOD) scores using a polygenic model adjusted for age and sex. **Results.** We identified multiple QTL for novel CVD biomarkers. For paraoxonase, a QTL was identified on chromosome 7q22-q31 (108-119 cM, maximum twopoint LOD=2.39, multipoint LOD=2.76); chromosome 8p12-p11 (51-59 cM, twopoint LOD=1.94, multipoint LOD=2.77); and chromosome 19p13-q12 (46-51 cM, twopoint LOD=0.91, multipoint LOD 2.18). Two of these QTL (7q, 19p) have been identified previously; the chromosome 8p locus is novel. For D-dimer, a QTL was identified on chromosome 9q33-q34 (128-149 cM, twopoint LOD=1.38, multipoint LOD=2.31), chromosome 15q12 (8-15 cM, twopoint LOD=0.65, multipoint LOD=2.00) and chromosome 3q22-q24 (140-152 cM, twopoint LOD=1.18, multipoint LOD=1.68). For GSP, two QTL were identified: chromosome 13q12-q13 (17-38 cM, twopoint 0.26, multipoint LOD 2.00) and chromosome 3p14 (84-94 cM, twopoint 0.53, multipoint 1.46). For CRP, modest QTL were identified on chromosomes 3q26-q27, 7q31-q34, 14q31-q32, 19p12-q13, and 22q11-q12 (LOD 1.55-1.85). **Conclusions.** We report multiple QTL for novel cardiovascular biomarkers in a large family with a burden of CVD reflective of the average United States population. Several strong candidate genes underlie these QTL and could be contributing to variability of these CVD-related biomarkers, thus mediating CVD risk.

Identification of osteoporosis susceptibility genes by computational disease gene identification strategy. *Q. Huang, G. Li, A. Kung* Dept Medicine, Univ Hong Kong, Hong Kong, Hong Kong.

We previously used five freely available bioinformatics tools (Prioritizer, Geneseeker, PROSPECTR and SUSPECTS, Disease Gene Prediction and Endeavour) to analyze the thirteen well replicated osteoporosis susceptibility loci and identify a subset of most likely candidate osteoporosis susceptibility genes (Huang et al. 2008). In current study, we experimentally tested the association between osteoporosis and the 9 most likely candidate genes [LAMC2(1q25-q31), MATN3(2p24-p23), ITGAV(2q31-q32), ACVR1(2q23-q24), TDGF1(3p21.31), EGF(4q25), IGF1(12q22-q23), ZIC2(13q32), BMP2(20p12)] which were pinpointed by all of 5 tools. Tag SNPs-based and gene-wide association studies were conducted in 1, 810 case-control Hong Kong Chinese. The cases were subjects with low bone mineral density (BMD) (Z-scores -1.28, equivalent to the lowest 10 percent of the population) at either the L1-4 lumbar spine or femoral neck. Control subjects had high BMD (Z-score +1.0) at the corresponding sites. A total of 40 tag SNPs were identified from the CHB panel of the phase II HapMap project, and genotyped using the high-throughput Sequenom genotyping platform. Allelic/genotypic and haplotypic associations were evaluated by Haploview and binary logistic regression. The SNP rs9585308 in the ZIC2 gene showed consistent genotypic/allelic associations with BMD at all sites measured ($p = 0.04$-0.002). The SNP rs10178256 in the MATN3 gene showed significant genotypic/allelic associations with total hip BMD and troancher BMD ($p = 0.05$-0.01). Interestingly, mutations in the MATN3 gene have been reported in a variety of skeletal diseases and a functional knockout of the MATN3 gene increases BMD in mice (van der Weyden, et al. 2006). Our results thus demonstrated the feasibility of a computational disease gene identification strategy for prediction of susceptibility genes that affect complex diseases such as osteoporosis.

Interstitial lung disease in two patients with Rubinstein-Taybi syndrome. *R. Kosaki[1], S. Kikuchi[2], G. Koinuma[2], M. Higuchi[2], K. Kawasaki[2], K. Kosaki[3]* 1) Div.Clin Genet & Mol Med, Natl Ctr Child Health & Dev, Tokyo, Japan; 2) Div. Pul , Natl Ctr Child Health & Dev, Tokyo, Japan; 3) Dept Ped, Keio Univ Sch Med, Tokyo, Japan.

Rubinstein-Taybi syndrome (RTS) is characterized by broad thumbs and great toes, short stature, mental retardation, and distinctive facial features including downslanting palpebral fissures, columella extending below the nares. CREBBP and EP300 are the causative genes for RTS. Here we report on two patients with RTS who developed interstitial lung disease. [Case 1] The patient was born after 38 weeks of gestation with a birth weight of 2840g. At age 6 weeks, he started to develop tachypnea and cyanosis. Based on a hazy ground glass appearance on the chest X-ray and elevated serum marker KL-6, he was diagnosed as having interstitial lung disease. After treatment with corticosteroids, his respiratory status improved. He had typical RTS phenotype and a complex CREBBP mutation involving a 6-base deletion and an 11-base insertion that truncates protein [Case 2] The patient was born after 37 weeks of gestation with a birth weight of 1943g. At birth he had PDA, congestive heart failure, and pulmonary hypertension. PDA was ligated at age 1 month. After age 4 months, he experienced several episodes of pneumonia that required occasional ventilatory support. At age 12 months, X-ray revealed a hazy ground glass appearance. Based on elevated serum KL-6, he was diagnosed as having interstitial lung disease. He died because of irespiratory failure. Dysmorphic features of the patient were compatible with RTS. To the best of our knowledge, development of interstitial lung disease has never been reported previously in relation to RTS. Nevertheless, the similarity of the clinical course of two patients herein reported suggests that patients with RTS may be susceptible to interstitial lung disease. Recurrent pulmonary infection, a relatively common complication of RTS, may have contributed to fibrotic process of the lung. Alternatively, mutations in CREBBP, a transcoactivator of various transcriptional factors, may have direct impact on the structural integrity of the alveolar wall.

Variation of gene silencing contributes in a variety of gene expression in mammalian cells. *H. Hohjoh, Y. Tamura*
Natl Inst Neuroscience, NCNP, Tokyo, Japan.

Gene expression complexity is required for gaining a variety of biological functions, regulations and morphology in organisms. MicroRNAs (miRNAs) are small noncoding RNA and play a role in gene expression regulation by inhibiting translation of their target messenger RNAs (mRNAs). In this study, we indicate contributions of endogenous miRNAs, as well as target mRNAs, in the generation of a variety of gene expressions. We investigated the effects of endogenous let-7 miRNA on the expression of target genes in various mammalian cells. The results indicate that different cells had different levels of gene silencing against the target genes, depending upon the level of let-7. In addition, we also identified the intriguing regulation of the High Mobility Group A2 (Hmga2) gene expression involving endogenous let-7 during neuronal differentiation of mouse embryonal carcinoma P19 and human teratocarcinoma NTera2D1 cells, where the mouse Hmga2 underwent gene silencing and the human HMGA2 escaped gene silencing by its alternative splicing. The evidence presented here suggests that various levels of gene silencing involving endogenous miRNAs and target mRNAs most likely yields immensely various and complex gene expression.

Molecular mechanisms for subtelomeric rearrangements associated with the 9q34.3 microdeletion syndrome. *S. A. Yatsenko[1], E. Brundage[1], E. K. Roney[1], S. W. Cheung[1], A. C. Chinault[1], J. R. Lupski[1,2,3]* 1) Dept Molec & Human Gen, Baylor Col Med, Houston, TX; 2) Pediatrics, Baylor College of Medicine; 3) Texas Children Hospital, Houston, TX.

We characterized genomic rearrangements in 28 unrelated patients with 9q34.3 deletions using a custom designed high-resolution microarray. Four distinct rearrangement categories were delineated: terminal deletions, interstitial deletions, derivative chromosomes, and complex rearrangements; each results in haploinsufficiency of the *EHMT1* gene and a characteristic phenotype. Interestingly, 25% of our patients had *de novo* interstitial deletions, 25% were found with derivative chromosomes and complex rearrangements, and only 50% were bona fide terminal deletions. In contrast to genomic disorders that are often associated with recurrent rearrangements, breakpoints involving the 9q34.3 subtelomere region are highly variable producing nonrecurrent rearrangements. Molecular studies identified 3 potential breakpoint cluster regions. Interspersed repetitive elements such as *Alu*, LINE, long terminal repeats and simple tandem repeats are frequently observed at the breakpoints. Such repetitive elements may play an important role by providing substrates with a specific DNA secondary structure that stabilize broken chromosome or assist in DNA DSB repair. Sequence analyses of the junction fragments suggest that subtelomere deletions can be stabilized by both homologous and nonhomologous recombination mechanisms, through a telomere-capture event, by *de novo* telomere synthesis, or multistep breakage-fusion-bridge cycles.

Genome Wide Linkage and Admixture Scans in African American Families from the American Diabetes Association GENNID Cohort. *S. C. Elbein[1], S. K. Das[1], D. M. Hallman[3], C. L. Hanis[3], S. J. Hasstedt[2]* 1) Endocrinology, Dept of Medicine, CAVHS and Univ Arkansas Med Sci, Little Rock, AR; 2) Dept of Human Genetics, University of Utah Health Sciences Center, Salt Lake City, UT; 3) Human Genetics Center, School of Public Health, Univ. of Texas Health Science Center , Houston, TX.

We examined 1496 individuals from 580 African American families ascertained from 10 centers. Participants were evaluated at 5,958 single nucleotide polymorphisms (SNPs). After data cleaning and removal of singleton and problematic pedigrees, analyses were conducted on 1344 individuals from 530 families (1293 affected sib pairs; 1021 full siblings, 306 parent-offspring pairs, 267 half sibs, 43 avuncular pairs, and 14 grandparent/grandchild pairs) using variance components analysis for type 2 diabetes (T2D), age of diagnosis (AOD), and BMI at 5619 SNPs. Ordered subsets were investigated based on AOD, BMI, waist circumference, waist:hip ratio, and European Admixture using nonparametric linkage (NPL)scores. The strongest signal for T2D was on chrm 2 at 127 cM and 108 cM (lod 4.53 and 2.98, resp), where AOD also showed suggestive linkage(1.66 at 115 cM). Chromosome 13p likewise showed linkage for both T2D and AOD (3 cM - 22 cM; lod 2.42 for T2D;2.46 for AOD). Suggestive T2D regions were chrms 4 (135 cM; lod 2.26), 7 (79 cM, 2.93), 11 (123 cM, 2.36), and 16 (56 cM, 2.43). The best AOD score lod 2.96 (chrm 18, 66 cM). Suggestive ordered subset scores included chrm 1 (30 cM; admixture), chrm 3 (11 cM; AOD), chrm 7 (131 cM; WHR); and chrm 14 (57 cM; T2D duration. Multipoint admixture linkage disequilibrium was calculated from 4486 markers spaced at >0.05 cM. The best admixture signal was found on chrm 12 at 90 cM (p=0.0003), which approached but did not meet genome-wide significance. The regions of best linkage (chrm 2, 13, 18) are gene rich (122, 27, and 47 genes, resp), and include candidate genes LIPG (18q), PDX1(13q), PAX8 (2q), IL18R1 (2q), and MAP4K4(2q). Secondary peaks on chrms 4 (125 - 135 cM), 7 (27-78 cM), and 22 (33 cM)overlapped with previous reports. Our results suggest several novel T2DM susceptibility genes, and particularly support a recent report on chromosome 7 in African-Americans.

Information-Theory methods for analyzing Gene-Gene and Gene-Environment Interactions involving quantitative traits. *L. Sucheston[1, 2], P. Chanda[1], S. Liu[1,2], A. Zhang[1], M. Ramanathan[1]* 1) State Univ New York, Buffalo, Buffalo, NY; 2) Roswell Park Cancer Institute, Buffalo, NY.

We developed two information theoretic metrics and a computationally efficient algorithm for analyzing genome wide data for evidence of gene-gene (GGI) and gene environment interactions (GEI) associated with quantitative traits (QT). The approach can be employed for case-control and case designs. We evaluated the power and type I error of the phenotype-associated information (PAI), which is obtained from the total correlation information (TCI) by removing the contributions for inter-variable dependencies (resulting from factors such as linkage disequilibrium) and the KWII, which is defined in terms entropies of both the individual variables and the entropies the combinations of the variables, using a series of controlled numerical experiments. In addition to these simulations, we examined the ability of the metrics when used in a greedy search algorithm to efficiently analyze genetic association with QT on a genome wide level using GAW15 data (generated by complex simulations based on a rheumatoid arthritis data). The metrics and search algorithm were also applied to a QTL mouse mapping study of HDL levels and atherosclerotic lesion size. The results of this analysis were compared to existing literature and a comparative genomics approach was used to evaluate the ability of the PAI and greedy search to replicate other study results, narrow regions and generate additional regions of interest. In addition to good power and type I error in the simulations, involving only a few SNPs, the information metrics were found to have excellent sensitivity at the genome level for identifying the GEI in the GAW15 data. Results from the mouse QTL analyses when used in a comparative genomics analysis showed that our method was capable of successfully identifying all published regions associated with HDL and atheroscelortic disease in humans and several that have only been found using fine mapping studies following a genome scan. These metrics and the algorithm provide a promising approach for analyzing the GGI and GEI associated with QT in an increasingly data rich age.

pHCR: A Parallel Haplotype Configuration Reduction Algorithm for Haplotype Interaction Analysis. *W. Makarasara[1,2,4], A. Assawamakin[3], A. Intarapanich[3], S. Fucharoen[2,4], N. Chaiyaratana[5], N. Kamatani[1], S. Tongsima[3]*
1) Center for Genomic Medicine, Institute of Physical And Chemical Research, RIKEN, Minato-ku, Tokyo, Japan; 2) Thalassemia Research Center, Institute of Science and Technology for Research and Development, Mahidol University, Nakornpathom, Thailand; 3) Biostatistics and Informatics Laboratory, Genome Institute, National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, Pathumthani, Thailand; 4) Department of Biochemistry, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand; 5) Department of Electrical Engineering, Faculty of Engineering, King Mongkuts Institute of Technology North Bangkok, Bangkok, Thailand.

Multiple genes and their interactions are believed to underlie most of complex diseases. Within the last decade, extensive studies have successfully identified disease-associated genetic variants. However, the lack of the knowledge for modeling the gene-gene interaction has led to the failure in the full explanation of the genotype-phenotype relationship. To overcome such an obstacle, we here introduce a novel algorithm for modeling the gene-gene interaction via haplotype interaction called Parallel Haplotype Configuration Reduction (pHCR). This technique extends the original multifactor dimensionality reduction (MDR) algorithm by utilizing haplotype contribution values (c-values) and a haplotype interaction scheme to achieve the goal of specifying the haplotype combination which best classified cases and controls. The parallel computing technology is introduced to handle the large scale analysis. Results using simulation data sets of twelve two-locus disease models suggested that pHCR can predict the gene-gene interaction correctly in most epistasis models. In addition, incorporation of haplotype which includes the linkage disequilibrium (LD) information showed an increase in power for detecting true interactions when susceptibility loci are not present in the data set and other SNPs are in weak LD with the susceptibility loci.

Meta-analysis of genome-wide association scans reveals new loci for body mass index in early adulthood. *S. I. Berndt[1], L. Qi[2], A. U. Jackson[3], M. Garcia-Closas[1], M. Boehnke[3], F. Hu[2], D. J. Hunter[2], S. J. Chanock[1], R. B. Hayes[1]*
1) Division of Cancer Epidemiology & Genetics, National Cancer Institute, Bethesda, MD; 2) Departments of Nutrition and Epidemiology, Harvard School of Public Health, Boston, MA; 3) Center for Statistical Genetics, Department of Biostatistics, University of Michigan, Ann Arbor, MI.

Obesity is an important risk factor for a multitude of diseases. Although obesity has high heritability, efforts to detect genetic loci associated with the trait have yielded a limited number of susceptibility genes. Considering the heterogeneity in obesity and that genetic determinants may be important particularly in early onset obesity, we conducted a meta-analysis of genome-wide association (GWA) scans from three studies (Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial, Nurses Health Study, and Finland-United States Investigation of NIDDM Study) including ~ 5,400 individuals of European ancestry to identify genetic loci associated with body mass index (BMI) in early adulthood (ages 18-20 years). Based on scans of 300-500K SNPs, each study imputed genotypes for the ~2.5 million common SNPs in the CEU HapMap and tested the association under an additive genetic model (1 d.f.). SNPs with a minor allele frequency <1% or low imputation quality score ($r^2 < 0.3$) were excluded, and a meta-analysis of the associations using the square root of the sample size as weights was performed. The recently identified variants in *FTO* and near *MC4R* were associated with BMI in early adulthood ($p = 0.01$ and $p = 0.02$, respectively). We discovered one novel locus on chromosome 4 with a p-value $< 1 \times 10^{-6}$, and nine loci not previously implicated in obesity showed nominally significant associations with p-values $< 1 \times 10^{-5}$. Some of these loci are located near genes related to metabolism or growth factors, which are promising candidates for obesity. We are currently replicating the top findings in an additional 10,000 persons of European ancestry. In conclusion, our study identified several new promising genetic variants associated with BMI in early adulthood and will hopefully shed additional insight into the complex etiology of obesity.

In-silico pharmacokinetic and cellular simulation of pharmacological chaperone therapy for GM2 gangliosidosis.
J. G. Keimel Biomedical Engineering, University of Minnesota, Minneapolis, MN.

Background: Inadequate -hexosaminidase A (HexA) activity results in an over-accumulation of GM2 ganglioside in neuronal lysosomes (lys) and disruption of normal cell function. G269S, the primary mutation associated with Adult Tay-Sachs Disease (ATSD), does not impact the viability of the HexA active site, but is believed to cause post-translation misfolding, instability of the HexA constituent monomers, and premature routing for degradation from the endoplasmic reticulum (ER). Pharmacological chaperone (PC) therapy has been proposed to improve HexA monomer stability in the ER and thereby increase the HexA dimer transport to the lys. Prior in-vitro studies have shown pyrimethamine (pyr) to be a potential PC therapy for ATSD, but being a HexA inhibitor, it also poses possible safety risks. **Model Description:** In lieu of a suitable animal model, a computer simulation has been developed to provide insight on pyr pharmacokinetics (PK) and GM2 response that might be observed in ATSD patients. The simulation uses three interlinked models. The PK model uses classical two compartment theory to estimate neuronal pyr concentration. An ER model modulates the enzyme stability based on the pyr level and predicts the relative amount of HexA transported to the lys. The third model simulates lysosomal GM2 degradation and HexA inhibition caused by pyr. The models are based on simultaneous solution of 18 ordinary differential equations and use previously published parameter values, when available. The lysosomal model incorporates an algorithm that increases HexA half-life as a function of GM2 substrate. It is hypothesized that this simple regulatory mechanism allows ATSD patients to remain in a rather stable disease state for decades. **Simulation Results:** Simulations of continuous daily pyr therapy results in a 40 day transient HexA increase but with a persistent decrease in GM2. The transient HexA level response suggests that an alternative measurement of GM2 levels may be required to assess long-term clinical efficacy. **Conclusions:** The simulation provides a means of creating hypotheses on PC mechanisms, but clinical studies are required for validation.

Crossing-over satellite and jumping SRY. *C. C. Lin[1,2], Y.-C. Li[3], S.-C. Chien[4,5], L.-J. Hsieh[1], F.-J. Tsai[5,6]* 1) Lab for Chromosome Research, Dept Medical Research, China Medical Univ & Hosp, Taichung, Taiwan; 2) Graduate Institute of Basic Medical Sciences, China Medical Univ, Taichung, Taiwan; 3) Dept Biomedical Sciences, Chung Shan Medical University, Taichung, Taiwan; 4) Dept Obstetrics and Gynecology, China Medical Univ Hosp, Taichung, Taiwan; 5) Dept Medical Genetics, China Medical Univ, Taichung, Taiwan; 6) Dept Pediatrics, China Medical University Hosp, Taichung, Taiwan.

We report here two extreme rare and unique prenatal diagnostic cases. The first case is a phenotypically normal male fetus with a satellited short arm of the Y chromosome, 46,XYps. Amniocentesis was performed due to advanced maternal age and parental chromosome study revealed that the father has a normal Y and a satellited short arm X chromosome, 46,XpsY. The mother has normal female karyotype. The couple has another son with normal male karyotype. This is the second documented case of Yps, while it is the first time to demonstrate the likely mechanism that a crossing-over between Xps and terminal short arm of the Y occurred during spermatogenesis, for production of Yps in this case. In the second case, amniocentesis revealed a male fetus with apparently normal female karyotype,46,XX. Surprisingly, FISH study with SRY probe detected a hybridization signal on the terminal short arm of a chromosome 3 of the fetus. An apparently normal male newborn was delivered. Both parents have the normal karyotype and the SRY locus located on the terminal short arm of the fathers Y chromosome. We hypothesize that instead of the more common translocation between Xpter and Ypter, a jumping translocation between Ypter involving the SRY locus and terminal 3p occurred during paternal meiosis. The fetus in this case had received a normal X and a SRY translocated chromosome 3 from a paternal gamete. To our knowledge, it is the first such a case reported.

A novel six nucleotide insertion and a novel nonsense mutation in the fructose-1,6-bisphosphatase 1 gene of patients with clinical diagnosis of the fructose-1,6-bisphosphatase deficiency. *H. Abalkhail[1], M. Faiyaz-Ul-Haque[1], M. Al-Owain[2], Z. Al-Hassnan[2], H. Al-Zaidan[2], Z. Rahbeeni[2], M. Al-Sayed[2], A. Balobaid[2], A. Cluntun[1], M. Toulimat[1], F. Al-Dayel[1], S. H. E. Zaidi[3]* 1) Pathology & Lab Med (MBC 10), King Faisal Special Hosp, Riyadh, Saudi Arabia; 2) Dept of Medical Genetics, King Faisal Specialist Hospital & Research Centre, Riyadh, Saudi Arabia; 3) Dept of Medicine, University Health Network & University of Toronto, Toronto, Ontario, Canada.

Fructose-1,6-bisphosphatase 1 (FBP1) is an important regulator of gluconeogenesis which catalyzes the hydrolysis of fructose 1,6-bisphosphate to fructose 6-phosphate and inorganic phosphate. FBP1 deficiency results in impaired gluconeogenesis which produces episodes of hyperventilation, apnea, hypoglycemia, and metabolic and lactic acidosis in patients. This autosomal recessive disorder is caused by mutations in the FBP1 gene which encodes for the fructose-1,6-bisphosphatase 1 protein. Although mutations in FBP1 gene have been reported in Japanese, European and North American populations, genetic causes of fructose-1,6-bisphosphatase deficiency in the Arab population have not been investigated. Here, we describe five consanguineous families in which patients were clinically diagnosed with the deficiency of fructose-1,6-bisphosphatase. Mutation analyses of the FBP1 gene identified a novel six nucleotide insertion in three families, and a novel stop codon mutation in two other families. The insertion mutation which encodes for two additional amino acids, may affect the FBP1 enzyme activity by altering its structure or allosteric regulation. The stop codon encoding mutation is expected to produce protein truncation in the active site region of the FBP1 protein. These novel mutations in the FBP1 gene are the first known genetic causes of the FBP1 deficiency in patients of Arab ethnicity. Reoccurrence of these mutations in other unrelated families provides a strong justification for analysis of Arab patients with clinical diagnosis of fructose-1,6-bisphosphatase deficiency for these mutations.

Canine polydactyl mutations with heterogeneous origin in the conserved intronic sequence of LMBR1. *C. Kim[1], K. Park[1], J. Kang[1], K. Subedi[1], J. H. Ha[2], C. Park[1]* 1) Biological Science, Korea Advanced Institute of Science and Technology, Daejeon, Korea; 2) Genetic Engineering, college of Natural Sciences, Kyungpook National University, Daegu, Korea.

Canine preaxial polydactyly (PPD) in the hind limb is a developmental trait that restores the first digit lost during canine evolution. Using a linkage analysis, we demonstrated previously that the affected gene in a Korean breed is located on canine chromosome 16. The candidate locus was further limited to a linkage disequilibrium (LD) block of less than 213 kb comprising the single gene, LMBR1, by LD mapping with single-nucleotide polymorphisms (SNPs) for affected individuals from both Korean and Western breeds. The ZPA regulatory sequence (ZRS) in intron 5 of LMBR1 was implicated in mammalian polydactyly. An analysis of the LD haplotypes around the ZRS for various dog breeds revealed that only a subset is assigned to Western breeds. Furthermore, two distinct affected haplotypes for Asian and Western breeds were found, each containing different single-base changes in the upstream sequence (pZRS) of the ZRS. Unlike the previously characterized cases of PPD identified in the mouse and human ZRS regions, the canine mutations in pZRS lacked the ectopic expression of sonic hedgehog in the anterior limb bud, distinguishing its role in limb development from that of the ZRS.

A Common Intronic Variant of *CXCR3* is Functionally Associated with Gene Expression Levels and the Polymorphic Immune-cell Responses to Stimuli. *Y. Kim[1], C. Choi[1], M. Hwang[2], C. Park[3], H. Nam[1], H. Chang[3], B. Han[1], K. Kimm[4], H. Kim[1], B. Oh[1]* 1) Ctr Genome Science,3rd Fl, KNIH, Seoul, Korea; 2) Graduate School of Medicine, Korea University, Seoul, Korea; 3) Genome Research Center for Allergy and Respiratory Diseases, Soonchunhyang University, Bucheon Hospital, Bucheon, Korea; 4) Merck Research Laboratories/WWL & ER/MSD Korea, Korea.

Background: Asthma is a complex trait disese, which renders the task of identifying its etiologic factors difficult. CXCR3 is a chemokine receptor that plays important roles in mediating chemotactic signals, and modulating the activation of lymphocytes. We have previously conducted a case-control study using a candidate gene approach to investigate the association of CXCR3 polymorphisms with the risk of asthma. Results from the epidemiological study showed that a common nucleotide variant in the CXCR3 intron (rs2280964G>A) was associated with disease susceptibility (1006 cases & 384 controls, OR=0.81 (0.69-0.94), P=0.007). Objective: The aim of our study was to evaluate the epidemiological study and provide functional evidence for the association of rs2280964G>A with asthma by investigating the effects of intronic variant on chemokine-mediated phenotypes of human-derived T cells. Methods: We used cell line-based in vitro and human primary T cell-based ex vivo studies to examine functional consequences of the intronic polymorphism with respect to the regulation of gene expression, splicing as well as immune responsiveness toward activating signals. Results: We present functional evidence indicating that the rs2280964A allele significantly correlates with decreased CXCR3 gene expression, which would lead to variation in immune cell responses to chemokine-cytokine signals in vitro and ex vivo, including a decrease in chemotactic activity. Conclusion: These findings, in conjunction with our previous epidemiological studies, may implicate a functional link between a common nucleotide variant of a chemokine receptor gene, CXCR3 and an etiology for a complex-trait disease, asthma.

Two mutations at the opposite sites in anticodon stem of mitochondrial tRNA-Trp gene cause different phenotypes on cytochrome c oxidase activity. *H. Hatakeyama, H. Komaki, Y. Goto* Dept. 2, Natl. Inst. Neurosci., Natl. Ctr. Neuro. & Psychiatry, Kodaira, Tokyo, Japan.

Various mutations have been found in mtDNA-encoded tRNA genes, and some frequently cause severe phenotypes with morphological and physiological alterations of mitochondria such as a3243g in tRNA-Leu(UUR) gene in MELAS. Here, we report homoplastic mtDNA mutations of c5541t and g5549a in the independent Japanese patients with mitochondrial disorders. The c5541t mutation was detected, for the first time, in the present study, these two mutations sit facing each other in the anticodon stem of tRNA-Trp gene. To identify the pathogenesis of these two mutations and the difference in phenotypic severity, we introduced cell-based functional screening system to patients mitochondria. Primary cultures from these two patients muscle were subjected to various biochemical and cell biological examinations. In comparison with a control group (n10), no significant differences were found in cellular ATP production and oxygen-consuming mitochondrial respiration. However, enzymatic analyses for mitochondrial respiratory chain complexes indicated the significantly decreased activities of both cytochrome c reductase and cytochrome c oxidase (COX) than those of controls. In addition, the normalized activity of COX by citrate synthase revealed the difference between these two mutations: c5541t mutation showed significantly reduced activity of normalized COX than that of controls, whereas g5549a mutation did not. We also confirmed the decreased activity of COX by histochemical staining; diffuse COX deficiency in the patient with c5541t mutation but only one COX-negative ragged-red fiber in the patient with g5549a mutation. If the disruption of base pair complementarity in the stem structure might be associated with tRNA dysfunction, our finding seems paradoxical because G-T base pair has more profound effect than A-C base pair. In summary, our newly found mutation of c5541t in tRNA-Trp gene would be causative to diffuse COX deficiency, and the g5549a mutation at the opposite site of c5541t mutation, which was previously described in only one patient, would also be milder effect on COX activity.

Molecular analysis of glucose-6-phosphate dehydrogenase deficiency in Saudi Arabian patients. *J. A. K. M. Bhuiyan[1], M. Faiyaz-Ul-Haque[1], R. Al-Nounou[1], A. Al-Abdullatif[1], A. Cluntun[1], T. Gonzales[1], M. Toulimat[1], F. Al-Dayel[1], S. H. E. Zaidi[2]* 1) Pathology & Lab. Medicine, King Faisal Specialist Hospital & Research Centre, Riyadh, Riyadh, Saudi Arabia; 2) Dept of Medicine, University Health Network & University of Toronto, Toronto, Ontario, Canada.

Glucose-6-Phosphate Dehydrogenase (G6PD) catalyzes an important step during conversion of glucose-6-phosphate to pentose phosphate. Deficiency of this enzyme produces hemolytic anemia in affected individuals. Other symptoms include neonatal jaundice, abdominal and/or back pain, dizziness, headache, dyspnea, and palpitations. G6PD deficiency is the most common human enzyme deficiency with an estimated 400 million peoples affected worldwide. This disorder is caused by mutations in the G6PD gene. More than 140 mutations have been identified with considerable variations in G6PD enzyme activities among various ethnic populations. Because of impaired malaria parasite growth in G6PD deficient red blood cells, G6PD deficiency confers a selective advantage in the geographical regions where malaria is common. Due to this beneficial effect and frequent consanguineous marriages, there is a higher incidence (15-26%) of G6PD deficiency in Saudi Arabia. The three G6PD mutations which are found in the Middle Eastern countries include the Mediterranean (S188F), the A- (V68M) and the Aures (I48T) variants. In the present study we have recruited approximately 1700 randomly selected individuals from Saudi Arabia. From this large group, 210 patients (133 men and 77 women) were confirmed for G6PD deficiency by enzyme assay. Their DNA was analyzed for the three mutations, which are common in this geographical region, by sequencing of the exons 3-7 of the G6PD gene. This study shows that the Mediterranean variant accounts for ~54% of the G6PD deficiency cases followed by the Aures variant in ~20% of the patients. The A- variant was rare (5%) in our cohort of patients. The 21% of G6PD deficient patients who were negative for these three mutations may carry less common variants in other regions of the G6PD gene. It is also possible that G6PD deficiency in these subjects is caused by mechanisms other than the G6PD gene mutation.

The common 677CT MTHFR and 66AG MTRR polymorphisms involved in folate metabolism are not risk factors for Down syndrome in a Danish population-based study. *M. Grigoriadou[1], H. Kokotas[1], A. Giannoulia-Karantana[2], M. B. Petersen[1]* 1) Department of Genetics, Institute of Child Health, Athens, Greece; 2) Department of Pediatrics, Athens University Medical School, Athens, Greece.

Chromosomal aneuploidy consists the leading cause of fetal death in our species. Around 50% of spontaneous abortions until 15 weeks of gestational age are chromosomally aneupoid, with trisomies accounting for 50% of the aneuploid abortions. Trisomy 21 is the most common chromosome abnormality in liveborns and is usually the result of nondisjunction of chromosome 21 in meiosis in either oogenesis or spermatogenesis. To investigate a possible relationship between folate metabolism and Down syndrome (DS), genetic polymorphisms in the methylenetetrahydrofolate reductase (MTHFR) and the methionine synthetase reductase (MTRR) genes were analyzed in the Danish population. Our cohort consisted of 181 mothers of children with DS versus 1,084 healthy controls. Polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) were used to examine the MTHFR 677CT and MTRR 66AG polymorphisms. No significant association between the two polymorphisms and the risk for DS was found. We conclude that the two common MTHFR 667TC and MTRR 66AG polymorphisms are not likely to be maternal risk factors for DS in our cohort and that the difference to previous studies is probably due to small sample size or geographic variation in gene polymorphisms involving gene-nutritional or gene-gene or gene-nutritional-environmental factors.

Strong linkage disequilibrium for the frequent GJB2 35delG mutation in the Greek population. *H. Kokotas[1], L. Van Laer[2], M. Grigoriadou[1], V. Iliadou[3], J. Economides[4], E. Ferekidou[5], S. Korres[5], A. Giannoulia-Karantana[6], G. Van Camp[2], M. B. Petersen[1]* 1) Department of Genetics, Institute of Child Health, Athens, Greece; 2) Department of Medical Genetics, University of Antwerp, Antwerp, Belgium; 3) Department of Psychoacoustics-Neurootology, AHEPA Hospital, 3rd Psychiatric Clinic, Aristotle University of Thessaloniki, Thessaloniki, Greece; 4) Department of Audiology-Neurootology, Aghia Sophia Childrens Hospital, Athens, Greece; 5) Department of Otorhinolaryngology, Athens University, Hippokration Hospital, Athens, Greece; 6) Department of Pediatrics, Athens University Medical School, Athens, Greece.

Up to forty percent of autosomal recessive, congenital, severe to profound hearing impairment cases result from mutations in the GJB2 gene. The 35delG mutation accounts for the majority of mutations detected in Caucasian populations and represents one of the most frequent disease mutations identified so far. Some previous studies have assumed that the high frequency of the 35delG mutation reflects the presence of a mutational hot spot, whilst other studies support the theory of a common founder. Greece is amongst the countries presenting the highest frequency of the 35delG mutation (3.5%), and a recent study raised the hypothesis of the origin of this mutation in ancient Greece. We genotyped 60 Greek deafness patients homozygous for the 35delG mutation for six single nucleotide polymorphisms (SNPs) and two microsatellite markers, mapping within or flanking the GJB2 gene, as compared to 60 Greek hearing controls. A strong linkage disequilibrium was found between the 35delG mutation and the DNA markers at distances of 34 kb on the centromeric and 90 kb on the telomeric side of the gene, respectively. A comparison of the present findings with those of a previous study from Belgium, UK and USA, demonstrated a common haplotype reflecting the common founder. Our study supports the hypothesis of a founder effect and we further propose that ethnic groups of Greek ancestry could have propagated the 35delG mutation, as evidenced by historical data beginning from the 15th century BC.

Sudden hearing loss in a family with GJB2 related progressive deafness. *M. Petersen[1], H. Kokotas[1], M. Theodosiou[2], M. Grigoriadou[1], G. Korres[2], E. Ferekidou[2], A. Giannoulia-Karantana[3], S. Korres[2]* 1) Department of Genetics, Institute of Child Health, Athens, Greece; 2) Department of Otorhinolaryngology - Head and Neck Surgery, Athens University, Hippokration Hospital, Athens, Greece; 3) Department of Pediatrics, Athens University Medical School, Athens, Greece.

Approximately one in 1,000 children suffers from severe to profound hearing loss (HL) at birth or during early childhood (prelingual deafness). Around fifty percent of prelingual deafness has been related to genetic causes. Among genetic non-syndromic deafness, autosomal recessive inheritance predominates, accounting for about 80% of the cases, and up to date about 50 genes have been identified. Mutations in the GJB2 gene are responsible for up to 40% of such cases in many populations. Around 90 GJB2 mutations have so far been reported to be associated with recessive, non-syndromic HL. One specific mutation, 35delG, has accounted for the majority of mutations detected in the GJB2 gene in Caucasian populations and consists of a deletion of guanine (G) in a sequence of six Gs leading to a frameshift and truncation of connexin 26, the GJB2 gap junction protein. Recent studies have described progression of HL in a proportion of cases with GJB2 deafness. We report an unusual family with four 35delG homozygous members, in which the parents were deaf-mute whilst both children had a postlingual progressive HL. Furthermore, the son suffered from an episode of sudden HL.

Analysis of OLR1 Polymorphisms across Human Populations. *I. M. Predazzi[1], C. Martínez-Labarga[2], L. Vecchione[1], R. Mango[3], C. Ciccacci[1], F. Amati[1], M. Crawford[4], O. Rickards[2], F. Romeo[3], G. Novelli[1]* 1) Univerisity of Rome Tor Vergata, Department of Biopathology; 2) Univerisity of Rome Tor Vergata, Department of Biology; 3) Univerisity of Rome Tor Vergata, Department of Internal Medicine; 4) University of Kansas, Lawrence, Laboratory of Biological Anthropology.

Genetic variants predisposing to complex diseases are an open question in medical and population genetics. Several studies demonstrated a correlation between cardiovascular disease (CVD) susceptibility and the genetic background of ethnic groups. Since different populations are subject to distinct environments, natural selection may produce population-specific allele frequencies. The human oxidized low density lipoprotein (lectin-like) receptor 1 (OLR1) gene is involved in the uptake of oxidised low density lipoproteins (ox-LDL) and in the pathogenesis of atherosclerosis. We have previously identified 7 different Single Nucleotide Polymorphisms (SNPs), 6 of them located within introns 4, 5, and 3' UTR (untranslated region), comprised in a linkage disequilibrium (LD) block strongly associated with the elevated risk to develop Myocardial Infarction (MI). In the present study we analyze the frequency distribution of two variants of OLR1 gene (rs3736235 and rs11053646) in 17 different populations from Europe, Asia, Africa and North America at genotypic and haplotypic level and we measure the apportionment of genetic diversity. The multivariate analysis of molecular variance (AMOVA), the correspondence analysis and the multidimensional scaling (MDS) representation of genetic distance matrix performed between the studied population samples highlight that the African populations are closer to the Asian populations, clearly separated from the Europeans. These results suggest a possible basis for varying susceptibility to CVD among groups correlated with the Out-of-Africa expansion or different level of stress.

Nonsense-mediated mRNA decay may be involved in the down-regulation of the *Alu*-containing splicing variants.
K. Inoue, K. Takano, Y. Goto Dept MR & BD Res, Natl Inst Neurosci, NCNP, Kodaira, Tokyo, Japan.

Although thousands of intronic *Alu* elements, the most abundant interspersed elements in human genome, have predicted capability to become alternative exons, only a small number of genes actually incorporate *Alu* elements as expressed splicing variants by unknown genomic basis. Here we tested our hypothesis that nonsense-mediated mRNA decay (NMD), an mRNA surveillance system by which mRNAs containing premature termination codons (PTCs) are selectively detected and disrupted, can serve as post-transcriptional eliminator of *Alu*-containing splicing variants (ASVs). Examination of the expression level of ASVs in 21 human genes revealed that ASVs are generally present as minor alleles in multiple human tissues as well as in HeLa and SH-SY5Y cells. Suppression of NMD in these cell lines resulted in upregulation of ASVs in some genes, suggesting a partial role of NMD in downregulation of the ASV expression. However, a serial introduction of PTCs in the non-truncating high-expresser ASV of the *ADARB1* gene failed to trigger NMD, suggesting that the efficiency of NMD on PTC-containing ASV can be variable and modulated by other factors. Computational prediction suggested that ASVs may possess the strength of splice sites comparable to other alternative exons and less number of exoninc splicing enhancers than constitutive or alternative exons. At individual gene level, no association between these predicted splicing scores with ASV expression level or sensitivity to NMD inhibition was noted. Together, our findings suggested that NMD may be involved in the down-regulation of the of the ASV expression together with other undetermined factors.

No evidence for skewed X-inactivation in fragile X syndrome premutation carriers. *L. Rodriguez-Revenga[1,2], I. Madrigal[1,2], C. Badenas[2], M. Milà[2]* 1) Centre for Biomedical Research on Rare Diseases (CIBERER), ISCIII, Barcelona, Spain; 2) Biochemistry and Molecular Genetics, Hospital Clinic, Barcelona, Spain.

X-Chromosome inactivation (XCI) is the mechanism by which gene dosage equivalence is achieved between female (XX) and male (XY) mammals. In the general female population, the X-inactivation process is random, and therefore XCI ratios have a normal distribution (with average of 50:50) with only a small percentage of females (5-8%) showing a skewed X-inactivation ratio (>90:10). Fragile X syndrome (FXS) premutation carriers (55-200 CGG repeats) do not present FXS symptoms but it has been shown that they have a higher risk of developing premature ovarian failure (POF) and/or fragile X associated tremor/ataxia syndrome (FXTAS). About 20% of the FXS female premutation carriers present POF and around 15% FXTAS. In order to evaluate if these pathologies are associated with skewed XCI patterns, we have studied the X-inactivation pattern in 270 FXS females carriers (41 POF, 2 FXTAS, 3 POF and FXTAS, and 224 no POF no FXTAS). Results showed that FXS permutation female carriers have a normal distribution and that there is no correlation with the CGG repeat number. On the basis of these observations we conclude that FXS female premutation carriers with or without POF and/or FXTAS do not present skewed X-inactivation and therefore, other molecular or environmental factors may predispose to these conditions. Acknowledgments: Marató TV3 (TV06-0810).

Genotype-phenotype correlations in two PWS patients carrying atypical deletions: additional evidence for a causative role of the HBII-85 C/D box snoRNA cluster. *L. Larizza[1,2], F. Malvestiti[1], L. Ballarati[1], G. Grugni[3], R. Pignatti[3], D. Giardino[1], M. T. Bonati[1]* 1) Lab. Citogenetica Medica e Genetica Molecolare, IRCCS Istituto Auxologico Italiano, Milano, Italia; 2) Lab. Genetica Medica, Ospedale S. Paolo, Universita di Milano, Italia; 3) Ospedale S. Giuseppe, IRCCS Istituto Auxologico Italiano, Piancavallo, Italia.

Prader-Willi syndrome (PWS) is a complex neurodevelopmental disorder clinically characterized by hypotonia and feeding difficulties in the neonatal period, failure to thrive in the postnatal period, hyperphagia in early childhood resulting in obesity, hypogonadism, mental retardation and behavioural difficulties. It is caused by the loss of function of genes mapped in 15q11-13 that are subject to genomic imprinting and expressed by the paternal allele only. Different genetic/epigenetic mechanisms can lead to PWS: approximately 70% of the patients carry a de novo deletion of paternal chromosome 15, 25% show maternal uniparental disomy 15, and the remaining 5% have imprinting defects or structural abnormalities involving chromosome 15. The vast majority of the 15q11-13 deletions shows recurrent breakpoints (bkps), with a single distal bkp and two proximal bkps. Refined bkps mapping allows to group the patients into two main common deletion classes: type I (TPI) and type II (TPII). We report on two patients carrying two different atypical deletions characterized by BAC FISH and array CGH (244K, Agilent). Both have been thoroughly analysed for clinical and behavioural characteristics using Wechsler Adult Intelligence Scale-Revised (WAIS-R) and Autism Diagnostic Observation Schedule (ADOS). Both patients show a classical PWS phenotype according to the clinical diagnostic criteria of Holm and a mild cognitive impairment. They carry yet unreported deletions smaller than TPI and TPII, sized about 4,3 Mb and 372 Kb. Within the latter deletion part of SNURF-SNRPN gene is mapped together with several C/D box snoRNA genes, among which the HBII-85 snoRNA cluster, that seems a likely candidate gene for PWS. This is the second reported PWS atypical deletion showing the potential causative role of HBII-85 genes in the PWS phenotype.

Molecular characterization by aCGH of a 2,8 Mb duplication at Xq26.3 in a male with mental retardation. *I. Madrigal[1,2], M. Fernandez-Burriel[3], L. Rodriguez-Bodi[1,2], M. Milà[1,2]* 1) Biochemistry and Molecular Gen, Hospital Clinic, Barcelona, Barcelona, Spain; 2) Unidad de Investigación. Hospital de Mérida. Badajoz; 3) Biochemistry and Molecular Genetics Department, Hospital Clínic and IDIBAPS.

Males with duplications in the distal long arm of the X chromosome are rare and in most cases are inherited from a carrier phenotypically normal. We report the clinical and molecular characterization of a Xq26.3 duplication in two brothers affected by MR. Chromosome analysis was normal and Multiplex Ligation Probe Amplification (MLPA) analysis detected a duplication of the ARHGEF6 gene inherited from a carrier mother. Both affected brothers presented moderate mental retardation and displayed dysmorphic features. Further characterization of the duplication by array CGH and FISH experiments with specific BAC probes, revealed a deletion of 28 contiguous BAC clones, spanning a region of 2,8 Mb in Xq26.3. X-inactivation studies in the mother showed a skewed X-inactivation (90/10) inactivating the X-chromosome inherited by the patient. Among the 20 genes included within the duplicated region we discuss the implication of ARHGEF6, PHF6, SLC9A6 and HPRT1 in the phenotype of the patient. Mutations or deletions in these four genes are responsible for syndromic and non-syndromic forms of mental retardation. Nowadays high-resolution technologies such as array CGH allow the detection of copy number aberrations in patients with MR. The characterization of these cryptic rearrangements is of clinical importance in order to provide a genetic counselling in carrier women for future pregnancies.

Fine delineated comparative genome mapping between Indian muntjac and human by cross-species BAC-FISH.

Y. C. Li[1], T. S. Li[2], P. C. Hsu[1], C. C. Lin[2] 1) Dept Biomedical Sci, Chung Shan Medical Univ, Taichung, Taiwan; 2) Department of Medical Research, China Medical University Hospital, Taichung, Taiwan.

Indian muntjac is a unique mammalian species with 2n=6/7 (the lowest chromosome number in mammals). The chromosome of this species was thought to have evolved through extensive chromosomal rearrangements by recent molecular cytogenetic studies. However, the molecular mechanism of chromosome restructure and gene order alteration during karyotypic evolution are not clearly elucidated so far. To clarify these issues, we established the highly resolution comparative map of the Indian muntjac-human using cross-species BAC-FISH mapping strategy in this study. We had previously constructed an Indian muntjacs BAC library with 4X genome coverage and established the highest density BAC-FISH map with 2081 muntjacs BAC clones on the Indian muntjac genome. In the present study, we selected 280 Indian muntjacs BAC clones to map onto the metaphase chromosomes of human and selected 300 human RPCI BAC clones to map onto Indian muntjac chromosomes. This mutual comparative FISH map would provide (1) the high resolution of the Indian muntjac-human comparative map, (2) the gene orders in the homologous synteny block to further deduce the rate of chromosome evolution, (3) more information on the accurate location of the evolutionary breakpoints and identifying the numbers of those breakpoints, and (4) shed more light on the genome organization of the species-specific genes.

Frequency of fragile X tremor ataxia syndrome in fragile X syndrome families. *M. Mila[1,2,3], I. Madrigal[1,3], J. Kulisevsky[4], J. Pagonabarraga[4], B. Gomez[5], L. Rodriguez-Revenga[1,3]* 1) Biochemistry and MolecularGenetics Department, Hospital Clinic, Barcelona, Spain; 2) IDIBAPS, Barcelona, Spain; 3) Centre for Biomedical Research on Rare Diseases (CIBERER), ISCIII, Barcelona, Spain; 4) Neurology Service, Hospital Sant Pau, Barcelona, Spain Centre for Biomedical Research on Neurodegenerative disease(CIBERNED), ISCIII, Barcelona, Spain; 5) Radiodiagnostic Service, Hospital Sant Pau, Barcelona, Spain.

Fragile X syndrome (FXS), which is caused by a CGG triplet expansion in the first exon of FMR1 gene, is the leading cause of familial mental retardation. Premutated individuals (55-200 CGG) do not present FXS symptoms, but approximately one third on them can present other manifestations such as a late onset ataxia /tremor syndrome (called FXTAS) or premature ovarian failure (POF).In order to determine the frequency of FXTAS among FXS grandparents, we have contacted by telephone with 151 families from 398 registered in our Service. These results evidence that FXTAS frequency among FXS families is around 12 per cent;. FXTAS frequency among permutated woman is 11 per cent; and FXTAS frequency among NTM is 25 per cent;. We obtain also information about thyroid dysfunction and fibromyalgia, about 20 per cent; of carrier women present at least one of them. We are now evaluating these patients psychologically, neurological and with magnetic resonance imaging. Molecular studies confirm a slight reduction of FMRP protein and increased levels of mRNA in these patients (x2-x5 folds). The description of associated pathologies (FXTAS and POF) to premutation carriers has modified genetic counseling for FXS, these two disorders and their consequences have to be taken into account by genetic counselor. Acknowledgements (Marató TV06-0810).

Paternal Isodisomy chromosome 6: clinical report. *A. Mur[1], L. Perez-Jurado[2], O. Garcia[1], M. Bonet[3]* 1) Sección Neonatología.Servicio de Peditria. Hospital del Mar UAB. Barcelona Spain; 2) Genetics Unit, Universitat Pompeu Fabra, Program in Molecular Medicine and Genetics, Hospital Vall dHebron, Barcelona, Spain. Centre for Biomedical Research on Rare Diseases (CIBERER), ISCIII, Barcelona, Spain; 3) Sección Endocrinologia.Servicio de Peditria. Hospital del Mar UAB Barcelona Spain.

Transient neonatal diabetes (TND; MIM 601410) is a developmental disease of insulin production with an incidence about 1 in 500,000 in Europe. TND is characterized by hyperglicemia occurring in the first days of life, glycosuria, dehydratation, severe intrauterine grow retardation and decreased adipose tissue. In about approximately 90% TND cases, chromosome 6 is involved, including paternal disomy, paternal duplications, or loss of maternal imprinting in 6p24. Mutations in ABCC8 and KCNJ11 genes located in chromosome 11 are also responsible for TND. No significant differences in clinical characteristics between patients with ABCC8 and KCNJ11 mutations are detected, whereas patients with a 6q24 abnormality have a significantly lower birth weight. We present here, a neonate female born after 37.4 week pregnancy to a healthy primipara. Familial antecedents show diabetes on fathers side. Neonate birth weight was 1,955g and she was 43cm long. Physical examination showed bilateral palpebral edema, macroglossia, umbilical hernia and abdominal distension. At 29hours of life she presented hyperglycemia without acidosis or ketosis. The patient presented anemia the second day of life, which required iron therapy and blood transfusion one moth after birth. Insulin treatment was started and maintained intermittently until 38 days of life. At 12 months she was normoglucemic with normal levels of insulin and peptide C. Genetic studies show a complete paternal uniparental isodisomy of chromosome 6.

***IronXS*: an Australian high school screening program for haemochromatosis.** *S. Metcalfe[1,2], M. Wolthuizen[1], E. Varley[1], V. Collins[1], I. Macciocca[3], MA. Aitken[1], L. Bond[1,2], K. Allen[1,4], M. Delatycki[1,3]* 1) Murdoch Childrens Research Institute, Melbourne, Australia; 2) Dept Paediatrics, The University of Melbourne, Melbourne, Australia; 3) Genetic Health Services Victoria, Melbourne, Australia; 4) The Royal Children's Hospital, Melbourne, Australia.

New research suggests that population genetic screening for hereditary haemochromatosis (HH), an easily preventable iron overload condition, should be reconsidered.[1] In the *HaemScreen* workplace-based study,[2] we found high uptake of screening (>90%) for people attending the information session, but only 6% of those eligible actually attended. This led us to consider other strategies for offering screening. We report our first year of data from *IronXS*, a screening program for the C282Y *HFE* mutation in high schools. A DVD-based information session was presented to 3840 year 10 and year 11 students at 19 schools. 1533 students had parental consent to participate (40% of eligible students; males:females = 45:55; mean age=15.7yr) of whom 1359 chose testing (35.4% overall uptake; 89% of attenders). This revealed 7 C282Y homozygotes (1 in 194), who were invited to clinic for genetic counselling, and 148 C282Y heterozygotes (1 in 9), who received their result in the mail. Students completed a baseline questionnaire (Q1). More than 90% of students answered all the knowledge questions correctly. A second questionnaire (Q2) was sent one month after results were received to all homozygotes and heterozygotes and a sample of wild-types (74% response rate). Knowledge retention was generally very high. There were no significant differences in general health perception scores, risk perception and anxiety levels between the three groups. Follow-up includes Q3 at 12 months and interviews with students, parents and teachers. Our first year data show that the education is highly effective and there have been no significant effects on psychological well-being and health risk perception of students who have been tested. Testing uptake is high for students with consent from parents, but this requirement appears to be a barrier to participation. We aim to screen 9000 students in total. [1]Allen et al NEJM 2008, [2]Delatycki et al Lancet 2005.

Copper supplementation restores Cytochrome c oxidase activity in a C.elegans model of COX deficiency. *E. Trevisson[1], C. Asencio[2], J. C. Rodriguez Aguilera[2], P. Navas[2], L. Salviati[1]* 1) Pediatrics - Genetics Unit, University of Padova, Italy; 2) Centro Andaluz de Biología del Desarrollo, Universidad Pablo de Olavide Sevilla,Spain.

Cytochrome c oxidase (COX) deficiency is one of the most frequent mitochondrial respiratory chain (MRC) defects in infancy and causes severe encephalomyopathy. Although it can be due to both mtDNA and nDNA mutations, most of these forms are inherited as autosomal recessive traits and are generally caused by mutations in nuclear genes encoding the ancillary proteins required for the assembly of the holoenzyme. COX19 and COX17 encode two closely related metallochaperones involved in copper delivery to the mitochondrial inner membrane that are required for COX assembly. We aimed at generating a multicellular model of COX deficiency using the nematode C.elegans. We down-regulated the expression of these copper chaperones through RNA interference in worms and analyzed their phenotype: the animals display a COX-dependent decrease in respiration and an increase in lactate production, recapitulating the phenotype observed in patients with mutations in other COX-assembly genes. They also show an increase in lifespan and reduced fertility, as it has been reported with other Mit mutants, nematodes carrying defects in genes involved in the biogenesis of the MRC. We analyzed whether copper supplementation could rescue the phenotype, as it has been demonstrated in fibroblasts of patients with mutations in the SCO2 copper chaperone. Our results indicate that in interfered nematodes, copper allows a partial recovery of the pathological phenotypes. In this work we showed that C.elegans proves to be a useful tool to investigate mitochondrial dysfunction observed in patients with MRC defects that often presents in a tissue-specific fashion; moreover, it allows studying potential pharmacological therapeutic strategies. We investigated the effect of copper supplementation on COX-deficient animals, which is specific for this kind of defect and could represent a realistic therapeutic option. To our knowledge, this is the first demonstration of the efficacy of copper supplementation in a multicellular system.

Assessment of polymorphic variation within VEGF and retinal angiomatous proliferation in neovascular age-related macular degeneration. *G. J. McKay[1], G. Silvestri[1], N. Orr[2], U. Chakravarthy[1], A. E. Hughes[2]* 1) Vision Science, Queen's University Belfast, N. Ireland, United Kingdom; 2) Medical Genetics, Queen's University Belfast, N. Ireland, United Kingdom.

Purpose. Recent development and use of VEGF inhibition therapy has been shown to offer improved treatment for those with neovascular AMD (nvAMD) and in particular, those with retinal angiomatous proliferation (RAP). This study seeks to determine whether VEGF is likely to be a major genetic contributor to nvAMD or specifically within a subset of patients with observed RAP lesions.

Methods. Haplotypic structure of the *VEGF* gene region was elucidated using HapMap data and tagged SNPs were identified. Polymorphisms were genotyped using SNaPshot technology (ABI) or by conventional DNA sequencing in an association study of 211 patients with end-stage nvAMD, which included a subset of 51 individuals identified with a RAP lesion and 238 age-matched disease-free controls. Association of polymorphic variation was assessed through logistic regression also measuring the effect of smoking status and variation within the genes *CFH* and *LOC387715* and RAP nvAMD.

Results. Logistic regression modelling, measuring the effect exerted by *CFH, LOC387715*, and smoking status, demonstrated significant association between rs3024994 and RAP nvAMD (Odds Ratio=3.0; 95% CI: 1.02-8.86; P value=0.046). This SNP was not associated with nvAMD where RAP lesions were undetected.

Conclusions. Previous studies have conflicted on the significance of variation within *VEGF* as a genetic contributor to the nvAMD disease process. Our results suggest that *VEGF* is not a major genetic initiator of AMD, but there is evidence to suggest that it may contribute to the formation of RAP lesions which appear to be more amenable to treatment with anti-VEGF therapies. While this study is the first to try and dissect the neovascular AMD phenotype in relation to association with genetic variation, the authors recognise that the sample numbers examined in this study are low and analysis of a much larger sub-phenotyped neovascular cohort is required.

An Imprinting Map of the Human Genome. *A. J. Sharp[1], H. Holster[2], E. Kriventseva[1], U. Surti[3], L. Iniguez[2], S. E. Antonarakis[1]* 1) Department of Genetic Medicine and Development, University of Geneva, Switzerland; 2) Roche NimbleGen, Inc., Madison, WI; 3) Department of Pathology, University of Pittsburgh, PA.

One of the major features associated with imprinting is the presence of parent-of-origin specific Differentially Methylated Regions (DMRs). Thus, the maternal and paternal genomes possess distinct epigenetic marks which distinguish them at imprinted loci. In order to construct an imprinting map of the human genome, we have profiled DNA methylation patterns genome-wide in rare uniparental tissues, utilizing a panel of Complete Hydatidiform Moles (CHMs), which have an exclusively paternal genetic contribution, and Ovarian Teratomas (OTs), which have an exclusively maternal genetic contribution. Methylated DNA was immunoprecipitated using anti 5-methyl cytidine and hybridized to Nimblegen high-density oligonucleotide tiling arrays composed of 21 million probes distributed throughout the genome, generating complete profiles of the paternal and maternal methylomes. Comparison of these profiles identifies numerous sites of parent-of-origin specific methylation, revealing a genome-wide imprinting map. Examination of known imprinted genes showed that the majority are associated with DMRs, validating this as an optimal system for the detection of imprinting. Furthermore, numerous candidate imprinted loci identified by previous sequence analysis were also associated with DMRs (Luedi et al. 2007 Genome Res 17:1723). Many novel putative imprinted regions on nearly every human chromosome were also identified. These include novel DMRs within known imprinted gene clusters, as well as chromosomal regions not previously thought to be imprinted, including several regions of common human aneuploidy. To validate our array data we performed bisulfite sequencing of putative DMRs, giving base-pair resolution of these imprints and confirming the presence of parent-of-origin specific methylation marks in multiple independent CHM and OT samples. Our data provides the first imprinting map of the human genome, demonstrates that the number of imprinted loci in the human genome is much higher than previously thought, and suggests that imprinting may influence the phenotypes of many human diseases.

The Currarino syndrome : two previously unreported nonsense mutations. *V. Benoit[1], S. Gaillez[2], T. Wester[3], Ch. Verellen-Dumoulin[1], P. Hilbert[1]* 1) Human Genetics Center, Institute of Pathology and Genetics, charleroi, Belgium; 2) Human Genetics Center, CHU, Liege, Belgium; 3) Akademiska Barnsjukhuset, Uppsala, Sweden.

The Currarino syndrome (CS) is characterized by 3 main clinical features : total or partial sacral agenesis with intact first sacral vertebra (sickle-shaped sacrum), anorectal malformation and a presacral mass (meningocoele or teratoma). This triad occurs as an autosomal dominant trait and is caused by mutations in the HLXB9 homeobox gene. If mutations are detected in only 50% of sporadic cases, the detection rate reaches 90% in familial cases. So far, 66 different mutations has been described, most of them being truncating mutations (57%). Only 7 nonsense mutations have been reported. In this study, sequence analysis of the HLXB9 gene was performed in 5 unrelated patients showing the clinical features of CS. No mutation was found in 2 patients, a known mutation (c.336dupG, p.P113fsX224) was identified in one patient and two previously unreported nonsense mutations were detected in the 2 last patients. The first, c.634C>T (p.Q212X), is sporadic and has been identified in a 2.5 year-old boy with anal stenosis, megarectum, anterior sacral myelomeningocoele and scimitar sacrum. The second, c.55C>T (p.R19X) is familial and affects 3 generations : a 6 month-old girl with anal stenosis, sacral dysplasia and presacral mass; her father, diagnosed at 8 months of age with presacral teratoma and anorectal stenosis and her paternal grandmother with sacral arachnoid cyst, operated at the age of 34 years. Phenotypic expression of the mutation varies within the family members, which is consistent with previous observations. These 2 novel truncating mutations confirm that haploinsufficiency is causing Currarino syndrome and indicate that patients with the triad should undergo genetic evaluation.

Genomic Sequence Analysis of the HMSN-P region on human chromosome 3q13. *S. Makino[1], K. Maeda[2], J. Jamiyansuren[1,2], K. Yasuno[3,4], R. Kaji[2], G. Tamiya[1]* 1) Division of Human Molecular Genetics, Department of Neurology and Neuroscience, The University of Tokushima Institute of Health Biosciences, Graduate School, Japan; 2) Department of Neurology and Neuroscience, The University of Tokushima Graduate School of Medicine, Japan; 3) Core Research for Evolutional Science and Technology (CREST), Japan Science and Technology Agency, Japan; 4) Department of Molecular Life Science, Tokai University School of Medicine, Japan.

Hereditary motor and sensory neuropathy with proximal dominancy (HMSN-P; MIM 604484) is endemic to Okinawa Islands, the most southern part of Japan, which is characterized by autosomal dominant inheritance, slowly progressive proximal muscle atrophy and weakness, sensory disturbance such as paresthesia and vibration loss, leading to be bedridden. The disease locus of HMSN-P has been mapped to 3q13-14. We previously reported our linkage study in a newly-found large family with many members developed the similar symptoms of HMSN-P in a western part of Japan around the HMSN-P locus identified in 3p13. However, the HMSN-P gene and an obvious causative mutation is still undiscovered. To find all variants within the HMSN-P region, we employed a genomic sequence analysis. We first constructed a BAC library using HMSN-P patient's genomic DNA. The insert size ranged from 35 to 175 kb with an average insert size of 102.0 kb. We screened the BAC library and obtained a chromosome-specific contig which covered 590 kb HMSN-P critical region also includes 7 genes and ESTs. Subsequently, we determined the entire genomic sequence of the HMSN-P region by the shotgun sequencing strategy with an average 5-fold redundancy. A comparison with reference sequence showed over 100 variants in the HMSN-P region, including many base substitutions, small indels and some chromosome rearrangements. This was twenty times as many as the number of variants reported previously. After listing these variants, we performed expression analyses, by northern hybridization and quantitative RT-PCR, for several candidate genes in which some desease-specific mutations of these variants were located. We would like to show these results in the presentation.

Two brothers presenting Early Infantile Epileptic Encephalopathy (EIEE) with the recently described mutation in the ARX gene. *S. Boulanger[1], L. Mariage[1], A. Destree[1], M. Foulon[2], P. Hilbert[1]* 1) Human Genetics Center, Institute of Pathology and Genetics, Charleroi, Belgium; 2) Department of neuropediatrics, CHU, Charleroi, Belgium.

Aristaless-related homeobox gene (ARX), is located within the human chromosome Xp22 region and is essential for the development of cerebral interneurons.Two recurrent polyalanine expansions in ARX are responsible for mental retardation and epilepsy in males. Recently, a new larger polyalanine expansion has been described - a 33-bp duplication in exon 2 of ARX gene expanding the original 16 alanine to 27 in the first poly A tract of the protein - it appeared de novo in two unrelated patients and caused Early Infantile Epileptic Encephalopathy (EIEE) with suppression-burst pattern. EIEE (also known as Ohtahara syndrome) is a severe and early form of epilepsy. The 33-bp duplication in exon 2 is the first gene alteration involved in idiopathic EIEE. We report two brothers presenting the same duplication with a typical phenotype of EIEE. Their parents are not bearing the mutation suggesting germinal mosaicism.

Meta-analysis of rheumatoid arthritis genome-wide association studies identifies common variants at *CD40* and five other gene loci. *R. M. Plenge[1,4], S. Raychaudhuri[1,4], E. F. Remmers[2], A. T. Lee[3], L. Gianniny[4], L. Padyukov[5], L. A. Criswell[6], C. I. Amos[7], M. F. Seldin[8], D. L. Kastner[2], T. W. J. Huizinga[9], N. de Vries[10], J. Worthington[11], M. Seielstad[12], R. E. M. Toes[9], E. W. Karlson[1], A. B. Begovich[13], L. Klareskog[5], P. K. Gregersen[4], M. J. Daly[4], BRASS, EIRA, GENRA, NARAC, WTCCC* 1) Harvard Medical School; 2) NIAMS, NIH; 3) Feinstein Institute; 4) Broad Institute; 5) Karolinska Institutet; 6) UCSF; 7) M.D. Anderson Cancer Center; 8) University of California, Davis; 9) Leiden University Medical Centre; 10) AMC/University of Amsterdam; 11) The University of Manchester; 12) Genome Institute of Singapore; 13) Celera.

BACKGROUND: Previous genetic studies have identified and validated risk alleles for autoantibody positive rheumatoid arthritis (RA): the MHC region, *PTPN22*, 6q23/*TNFAIP3*, *STAT4*, and *TRAF1-C5*. We hypothesize that additional common alleles of modest effect size remain to be discovered, and that these alleles can be identified through combining genome-wide association studies (GWAS) followed by large-scale replication. **METHODS**: We performed a meta-analysis of three recently published GWAS totaling 3,393 cases and 12,462 controls. We examined a set of 340,000 SNPs genotyped on the Affymetrix 500K platform as part of the WTCCC and imputed with Illumina case-control GWAS data from Sweden (EIRA) and North America (NARAC). The top 31 SNPs with P0.0001 but not previously associated with RA were genotyped in 3,929 autoantibody positive RA cases and 5,807 matched controls. **RESULTS**: We identified a reproducible and highly significant association to a common variant at the *CD40* gene locus (rs4810485, P=0.0032 replication, P=8.2x10^{-9} overall, OR=0.87). We also identified significant associations at five additional loci containing genes of immunological relevance: 9p13/*CCL21* (P=2.8 x 10^{-7}), 12q13/*PIP4K2C* (P=8.8 x 10^{-8}), 1p36/*TNFRSF14* (P=1.1 x 10^{-7}), 7q21/*CDK6* (P=4.0 x 10^{-6}), and 10p15/*PRKCQ* (P=4.4 x 10^{-6}). **CONCLUSIONS**: A novel variant at the *CD40* locus is definitively associated with RA. Along with other confirmed associations near *TRAF1* and *TNFAIP3*, this implies a central role for the *CD40* signaling pathway in RA pathogenesis.

The Human Genetics Historical Library: an International Resource. *P. S. Harper[1], K. Pierce[2], P. Keelan[2]* 1) Inst Medical Genetics, Cardiff University, Cardiff, United Kingdom; 2) Special Collections Cardiff University Library, Cardiff University, Cardiff, United Kingdom.

Historical and non-current scientific books are increasingly often dispersed or destroyed, even though, unlike journals, digitisation does not provide a satisfactory alternative for their preservation and is unlikely to do so in the near future. The Human Genetics Historical Library, initiated in 2003, provides a definitive collection of books on and related to human and medical genetics. Curated by Cardiff University Special Collections, it now contains over 2000 volumes, almost all donated by individual workers or institutions. A continuously updated list is available on www.genmedhist.org/HumanHistLib. The collection, as it grows, will provide an increasingly complete backdrop to the development of human genetics internationally during the past century, as well as a source for specific books. Detailed cataloguing, funded by Wellcome Trust, allows searching by category and is accessible on the web through the Voyager Library Catalogue (http://library.cardiff.ac.uk/). Further donations are welcome; please contact PSH or PK by email (above).

FLJ human cDNAs cloned focusing on mRNA variations and found some genes which predicted to have close relations between mRNA variations and diseases. *T. Isogai[1,2], A. Wakamatsu[1], J. Yamamoto[2], K. Kimura[3]* 1) Grad Sch Pharmaceutical Sci, Univ Tokyo, Tokyo, Japan; 2) Reverse Proteomics Research Institute, Tokyo, Japan; 3) Central Research Laboratory, Hitachi Ltd., Tokyo, Japan.

Human gene number was predicted to be about 20 thousand. But the number of the mRNA was predicted to be several times of gene number. Those varieties were thought to be caused by variations of splicing and transcription start site (TSS). In our NEDO human cDNA project, we sequenced about 44 thousand of human full-length cDNAs (FLJ cDNAs) and also obtained about 1.5 million of 5'-end onepass sequences (5'-EST), about 500 base length, of full-length cDNAs from about 100 kinds of cDNA libraries consist of human tissues and cells constructed by oligo-capping method. The majority of the insert size of those was over 2 kb and the full-length rate of 5'-end of those was 90%. All sequences of those were deposited to DDBJ/GenBank/EMBL and we constructed FLJ Human cDNA database, http://flj.hinv.jp/. Then about 11 thousand of full-length sequenced FLJ cDNAs, which was selected focusing on variations of splicing and TSS, was obtained from our cDNA resources described above. The sequences will be publicly available in a few months. In addition, we found some splicing and TSS variations which showed specific expression from newly isolated cDNAs. In order to search mRNA diversity, we analyzed gene expression profiles with the about 1.5 million of 5'-EST by oligo-capping method and then selected two types of genes mainly. The first: specific expression profiles; the second: variations of motifs and/or domains in coding regions. Using 8,313 cDNAs of 5,530 clusters from about 11 thousand of the newly isolated cDNAs, we selected 316 cDNAs of 144 clusters for further expression analyses by realtime PCR. As a result, 13 cDNAs of 8 clusters were shown tumor tissue specific expressions, and 20 cDNAs of 13 clusters were shown synovial, osteoclast, and/or blood cell specific expressions. This work was supported by a grant from New Energy and Industrial Technology Developmental Organization (NEDO) project of the Ministry of Economy, Trade and Industry of Japan.

Heritable somatic methylation and inactivation of *MSH2* in families with Lynch syndrome due to deletion of the 3 exons of *TACSTD1*. *M. J. L. Ligtenberg[1,2], R. P. Kuiper[1], T. L. Chan[3,4], M. Goossens[2], K. M. Hebeda[2], M. Voorendt[1], T. Y. H. Lee[3], D. Bodmer[1], E. Hoenselaar[1], H. G. Brunner[1], A. Geurts van Kessel[1], S. T. Yuen[3,4], J. H. J. M. van Krieken[2], S. Y. Leung[3,4], N. Hoogerbrugge[1]* 1) Human Genetics, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands; 2) Department of Pathology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; 3) Hereditary Gastrointestinal Cancer Genetic Diagnosis Laboratory, Department of Pathology, The University of Hong Kong, Pokfulam, Hong Kong; 4) Hereditary Gastrointestinal Cancer Registry, Department of Pathology, St. Pauls Hospital, No 2 Eastern Hospital Road, Causeway Bay, Hong Kong.

Lynch syndrome patients are susceptible to colorectal and endometrial cancers due to inactivating germline mutations in mismatch repair genes, including *MSH2*. Here we describe patients from Dutch and Chinese families with MSH2-deficient tumors carrying heterozygous germline deletions of the last exons of *TACSTD1*, a gene directly upstream of *MSH2* encoding the epithelial cell adhesion molecule Ep-CAM. The smallest deletion, which is detected in 9 patients from 4 families, encompasses only 4.9 kb and is located 14.5 kb upstream of *MSH2*. Due to the deletions transcription of *TACSTD1* extends into *MSH2*. The *MSH2* promoter *in cis* with the deletion is methylated in Ep-CAM positive, but not in Ep-CAM negative, normal tissues, thus revealing a correlation between activity of the mutated *TACSTD1* allele and epigenetic inactivation of the corresponding *MSH2* allele. Gene-silencing by transcriptional read-through of a neighboring gene as demonstrated here for *MSH2*, appears to represent a general mutational mechanism. Depending on the expression pattern of the neighboring gene that lacks its normal polyadenylation signal, this may cause either generalized or mosaic patterns of epigenetic inactivation, that are inherited over generations.

Exploration of Pathophysiological Mechanisms in Lamin A/C Associated Charcot-Marie-Tooth Disease (CMT2B1). *Y. Poitelon, C. Baudot, T. Hamadouche, N. Levy, V. Delague* Inserm UMR_S 910, Faculté de la Timone, Marseille cedex 05, France.

Lamins, a class of intermediate filaments, are major components of the nuclear lamina, a filamentous network underlying the inner face of the nuclear membrane. A-type Lamins are encoded by the same gene: LMNA, regulated by the AP1 complex (c-Fos & c-Jun). Up to date, eleven pathologies, described as laminopathies, have been associated with mutations in LMNA. One of these, Charcot-Marie-Tooth disease (CMT), type 2B1, is an autosomal recessive form of axonal CMT caused by the c.892C>T transition in LMNA exon 5. (p.Arg298Cys). In order to progress towards understanding of the pathophysiological mechanisms underlying CMT2B1, we studied two different models for the disease: human cells from patients homozygous for the c.892C>T mutation, and a Knock-In LmnaR298C/R298C mouse model. Gene expression studies performed on human microfluidic plates (Low Density Arrays) evidenced significant decrease in expression levels of several genes, including LMNA. These observations were confirmed in mouse brain, skeletal and cardiac muscle, sciatic nerve and spinal cord at the transcriptional level, as well as on lymphoblastoid human cell lines at the protein level. The p.Arg298Cys mutation lies within a coil-coiled domain, an important functional domain for intermediate filament polymerization. In silico predictions are in favor of a potential destabilizing effect of the mutation. Moreover, previous publications have shown that Lamins interact with c-Fos, a Leucine Zipper transcription factor, through their coiled-coil domain.We therefore propose a two-hit pathophysiological mechanism model: - The pArg298Cys mutation might destabilize complexes between A-type Lamins and transcription factors: the latter might be either components of the AP1 complex, or some nerve specific transcription factors, which remain to be identified. - LMNA seems to be autoregulated through an A_type Lamins - AP1 complex, which might be disrupted at the DNA level by the presence of the mutation. We are actually conducting further experiments in order to confirm these hypotheses.

A Haplotype-Specific Developmental Shift in Bitter Taste Sensitivity. *F. Duke, D. Reed, Y. Pepino, J. Mennella*
Monell Chemical Senses Center, Philadelphia, PA.

The purpose of this study was to define the effects of alleles within the haplotypes of the bitter receptor gene TAS2R38 and to investigate the interaction of haplotype and age on human bitter taste perception. A racially diverse sample of 980 people, ranging in age from 3 to 55 years old, were phenotyped for taste thresholds of a bitter compound (6-n-propylthiouracil; PROP) using methodologies that were validated for use with young children. DNA was extracted from cheek cells and genotyped for the three common alleles of the TAS2R38 gene (A49P, V262A, I296V). Subjects were grouped by diplotype and age (children, adolescents and adults) and compared for PROP thresholds. Eighty-five percent of all haplotypes observed were of one of two configurations, AVI (bitter insensitive) or PAV (bitter sensitive) while 15% were rarer haplotypes (i.e., AAI, AAV). The frequencies of some haplotypes differed between racial/ethnic groups, specifically, the AAI haplotype was found more often in Blacks whereas the AVI and AAV haplotypes were found more often in Whites. However, no differences between Black and White subjects within a diplotype or between males and females in PROP sensitivity were observed (all p-values >0.05). We found that children were more sensitive to PROP than adults but this observation was dependent upon diplotype. In particular, heterozygous children could perceive a bitter taste at lower PROP concentrations than could adults with the same diplotype, and the thresholds of adolescents were intermediate between adults and children (AVI/PAV; N=358; $p<0.001$). However these age differences were not apparent for subjects with either of the other common homozygous diplotypes (N=342; $p>0.05$; PAV/PAV and AVI/AVI). Biter sensitivity to the compound PROP is determined by the combination of the three alleles within the TAS2R38 gene and is also influenced by age, with children being more sensitive than adults. However not all diplotypes were affected to the same extent by development. The timing of the shift from child-like to adult-like bitter perception for groups with age-sensitive diplotypes occurred during adolescence.

A case of Recurrent Spontaneous Abortions with centric fission of chromosome 5. *M. Tanwar[1], M. Kumar[1], S. Mittal[2], D. Pathak[1], S. Venkatesh[1], M. B. Shamsi[1], R. Kumar[1], R. Dada[1]* 1) Lab. for Molecular Reproduction and Genetics, Deptt. of Anatomy, AIIMS, New Delhi, India; 2) Deptt. of Obs. & Gynae. AIIMS, New Delhi, India.

Centric fission is a rarely reported chromosomal abnormality in humans. Centric fission results when metacentric or submetacentric chromosome splits at the centromere giving rise to two stable telomeric products. We report a centric fission of chromosome 5 in a healthy female suffering from recurrent miscarriages. A couple was referred for cytogenetic analysis with bad obstetric history. Pregnancy history, age, occupation, disease information and all other medical records were reviewed. The wife had history of three abortions. The etiology of recurrent abortions is often unclear and may be multifactorial. Couples having multiple miscarriages are at risk for carrying chromosomal abnormalities. Majority of the studies have shown that most of the chromosomal abnormalities associated with recurrent abortions are numerical and structural which may adversely affect embryonic development but there are reports of recurrent miscarriages due to centric fission of the chromosomes. Peripheral blood was collected and lymphocyte cultures were set up for chromosomal analysis. Atleast 20 metaphases were analysed using GTG banding. The husband was cytogenetically normal and wife had 46,XX,-5,+fis(5)(p10),+fis(5)(q10) chromosomal complement. Both fission products were mitotically stable. To the best of our knowledge this is the first report on centric fission of chromosome 5 as the individuals with stable centric fission products have rarely been documented in humans. In this case, the centric fission does not cause any significant dysmorphic features and mental handicap. But heterozygous individuals for centric fission appear to be at increased risk for production of unbalanced gametes which consequently increased risks for spontaneous abortion, stillbirth and live born infants. However, even between homologous chromosomes, the size of centromeres is different from each other. A further study including more such cases is required to understand the etiology of recurrent spontaneous abortions in detail.

Accurate determination of copy-number-variations (CNVs): application to the alpha- and beta-defensin repeat region. *H. Nuytten[1], I. Wlodarska[1], S. Vermeire[2], JJ. Cassiman[1], H. Cuppens[1]* 1) Human Genetics, K.U.Leuven, Leuven, Belgium; 2) Gastroenterology, K.U.Leuven, Leuven, Belgium.

In the human genome, a considerable large number of genomic regions are found which are repeated several times. These regions are known as CNVs (Copy-Number-Variations) and may be polymorphic. There is an interest in determining the number of repeats in CNVs in individuals. We therefore developed a strategy that accurately determines the number of repeats in a CNV. Specifically, we developed an assay for the alpha- and beta-defensin repeat region, but any CNV can be characterized in an analogous way. For an accurate determination of the number of repeats, appropriate controls are needed, which are generally obtained by mixing different amounts of the (repeated) fragment under investigation and a reference fragment. Pipetting errors, and variable DNA concentration determinations, may interfere in such assays. We therefore developed concatemeric control constructs: each of these constructs contains 1 copy of a reference fragment in concatenation with a fragment of the repeated region. Different concatemeric control constructs were developed, each of them having a different number of the repeated region in concatenation. These concatemeric control constructs are then used for the generation of a standard curve, e.g. in a real-time PCR assay. We developed 4 concatemeric constructs with 1 copy of *DEFB1* and 1-4 copies of *DEFB4*. We analogously developed constructs for *DEFB104* and *DEFA1A3*. *DEFB4* and *DEFB104* are both located in the same CNV. In a real-time PCR assay, a correlation of R^2=0.98 was found between the number of repeats and the PCR yield. Using these controls as standards, the number of defensin repeats could then be accurately determined in DNA samples of 427 individuals. Each individual was tested three times, and each of these 3 experiments was performed in duplo. The copy number determined with the *DEFB4* and *DEFB104* was the same (SD = 0.2). We also validated the Real-Time PCR assay with interphase FISH. In conclusion, we have developed an accurate method to determine large scale copy number variation.

The Pompe Registry: Tracking Pompe Disease Symptoms in a Broad Patient Population. *B. Byrne[1], L. Case[2], P. S. Kishnani[3]* 1) Congenital Heart Center University of Florida, College of Medicine Gainesville, FL; 2) Div. of Physical Therapy, School of Medicine, Duke University Medical Center, Durham, NC; 3) Div. of Medical Genetics, Duke University Medical Center, Durham, NC.

Pompe disease (acid maltase deficiency) is a rare, progressive, and often fatal metabolic myopathy, which manifests as a clinical spectrum that varies with respect to age at onset, rate of disease progression, and extent of organ involvement. The underlying pathology is a deficiency of acid alpha-glucosidase (GAA). To gain a better understand of the natural course of Pompe disease, a global, voluntary, observational Registry was developed to collect anonymous, longitudinal data. As of September 2007, 400 patients from 23 countries are enrolled; 71% are Caucasian. Europe and North America enroll 85% of patients. For infants (n=78, 20%), the median age at symptom onset was 2.0 months and median age at diagnosis was 4.0 months. For adults (n=238, 60%), the median age at symptom onset was 26.3 years and median age at diagnosis was 34.5 years. Patients currently 18 years old (n=259) report the following symptoms most frequently: muscle weakness [lower extremities (81%), upper extremities (71%), trunk (57%)]; shortness of breath after exercise (61%) and at rest (33%); dependence on respiratory support (39%); sleep disturbance/apnea (37%); orthopnea (34%); and scapular winging (31%). Of the patients genotyped, 54% (76/140) expressed the IVS1-13T>>G mutation. These results show a significant delay from symptom onset to diagnosis in adult patients and highlight the need for greater disease awareness. As the Pompe Registry matures, data on prevalence and age at onset of symptoms in various patient subgroups may allow physicians to identify patients at earlier stages of disease progression; thus enabling therapeutic intervention before irreversible muscle damage occurs.

C4 gene copy number correlates to C4 protein levels and associates with development of SLE: results from Swedish study. *E. Lundstrom[1], Y. L. Wu[2], E. Svenungsson[1], I. Gunnarsson[1], A. Larsson[3], C. Y. Yu[2], L. Padyukov[1]* 1) Dept Medicine, Karolinska Inst & Hosp, Stockholm, Sweden; 2) Pediatrics; Molecular Virology, Immunology and Medical Genetics, The Ohio State University, and Center for Molecular and Human Genetics, The Research Institute at Nationwide Children's Hospital, Columbus, Ohio, USA; 3) Department of Clinical Chemistry and Pharmacology, Inst Med Sci, Akademiska Hospital, Uppsala, Sweden.

Objective: Copy number polymorphism (CNP) is one of many possible sources of phenotypic heterogeneity and susceptibility to human complex diseases. We analyzed association between CNP of the C4A and C4B genes at human chromosome 6p22 and systemic lupus erythematosus (SLE) in a Swedish cohort and investigated the correlation of the CNP with serum levels of C3, C3d and C4 in patients with SLE. Methods: 160 SLE patients and 180 controls matched on sex and age were included in the study. C4 CNP was discriminated by using RT-PCR and serum levels of C3, C3d as well as C4 were measured in patients by rate nephelometry. Correlation between the number of gene copies and the disease was analyzed by Chi-square test and complement factors levels were compared by Mann-Whitney test. Results: Our data show an increased odds ratio (OR) for SLE in carriers of 2 copies of C4A (OR: 2.26 95% CI: 1.37-3.74) or 4 copies of C4 total (C4A+C4B) (OR: 2.28 95% CI: 1.44-3.62). No association between C4B CNP and SLE was observed. Further analyses revealed a correlation between low copy numbers (4 copies) and low serum levels of C4 (p-value: 0.018). However, individual levels of C4 protein in serum of SLE patients with normal number of copies of C4 genes are in common range of concentration. No correlation between C4 CNP and serum levels of C3 and C3d was observed. Conclusion: Our data confirm, in Swedish population, previous findings of correlation between low copy number of C4 with low concentrations of C4 protein in serum and confer susceptibility to SLE in these individuals. Thus, a subgroup of SLE could be determined by C4 deficiency and further functional studies of mechanisms related to complement system are warranted for such subgroup.

Paraoxonase (PON1) gene polymorphisms in Fabry disease: Correlation with renal disease. *T. Shemesh[1], C. Whybra[3], S. Delgado-Sanchez[3], M. Beck[3], D. Elstein[2], G. Altarescu[1]* 1) Medical Genetic Unit, Shaare Zedek Medical Center, Jerusalem, Israel; 2) Gaucher Clinic, Shaare Zedek Medical Center, Jerusalem, Israel; 3) Metabolic Unit, the Universitäts-Kinderklinik2, Mainz, Germany.

Background: Fabry disease, an X-linked disorder due to decreased alpha-galactosidase-A activity, is associated with early-onset stroke, cardiomyopathy, and renal failure. There are very few markers to predict disease progression and hence we have attempted to develop polymorphic genotypes of symptomatic patients using genes that are predictive of specific disease signs. The two common polymorphisms of paraoxonase gene (PON1), Leu(55)Met and Gln(192)Arg are hypothesized to modulate cardiovascular risk as well as prognosis of stroke and deterioration of renal function in other diseases. PON1 is a high-density-lipoprotein-associated enzyme that apparently mitigates oxidative damage. Methods: DNA was extracted and PON1 polymorphisms performed by restriction enzyme analysis. The disease-specific Mainz Severity Score Index (MSSI) was calculated for patients. Results: Eighty patients (58 female) and 46 healthy controls (23 female) were evaluated for these 2 polymorphisms. There was a significant difference by chi-square analysis (p=.04) of the PON55 LL genotype among patients but no significant differences were noted with any PON 192 polymorphic genotypes. The PON55 LL genotype was strongly correlated with the MSSI renal sub-score categorized for more severe manifestations (p=.003); the omnibus test of model coefficients (p=.004) and the 2-way ANOVA (p=.0001) between groups for the LL genotype of PON55 were also predictive of more severe renal MSSI sub-scores, but not for cardiac, neurological, or general manifestations. Although there was no statistically significant difference between groups in levels of alpha-galactosidase-A activity, the LL genotype, which is correlated with higher PON1 activity, had the lowest levels. Conclusion: The PON55 LL genotype, i.e. higher PON1 activity, was significantly more common among patients with Fabry disease than controls and was correlated with more severe clinically relevant renal sub-scores as seen in other kidney diseases.

Polymorphisms and mutational analysis of the NOTCH3 gene in a large cohort of patients affected by leukoencephalopathy. *C. Ungaro[1,2], F. L. Conforti[1], T. Sprovieri[1], P. Servillo[1], M. Liguori[1], L. Citrigno[1], A. L. Gabriele[1], A. Magariello[1], A. Patitucci[1], M. Muglia[1], R. Mazzei[1]* 1) ISN-CNR, Mangone (CS), Italy; 2) Department of Neurosciences, Psychiatric and Anesthesiological Sciences, University of Messina, Messina, Italy.

CADASIL is a cerebrovascular disease caused by mutations in the NOTCH3 gene. Most CADASIL associated mutations result in a gain or loss of a cysteine residue in one of the 34 EGF-like repeats in the extracellular domain of the Notch3 protein, thus sparing the number of cysteine residues within a domain. To date, more than 130 different mutations in the NOTCH3 gene have been reported in CADASIL patients, the 95% being missense point mutations. Many polymorphisms have also been identified in the NOTCH3 coding sequence some of them leading to amino acid substitutions. The aim of the present study was to analyze the coding region and intron-exon boundaries of the NOTCH3 gene in a large cohort of patients affected by leukoencephalopathy to investigate the presence of genetic variants. We analyzed exons 3-4, exons 3-4-6-8 and exons from 2 to 23, respectively in 684, 542 and 157 patients affected by leukoencephalopathy. The molecular analysis revealed several nucleotide alterations in comparison to the wild type sequence; in particular, we identified 20 different mutations in 47 subjects from 30 families, 22 polymorphisms, 7 of them were novel, and 8 genetic variants of unknown pathological significance never been reported previously. In conclusion, this NOTCH3 gene mutational analysis performed in such a significant number of unrelated and related patients may help in molecular screening for NOTCH3 gene and may contribute to enlarge the NOTCH3 gene variation database. Acknowledgements We thank Drs G Guidetti, D Consoli, A Gambardella, A Toscano, for helping identify patients. The work was in part supported by the Italian Ministero dell'Istruzione, dellUniversità e della Ricerca Grants MIUR-FIRB2006-RBIP06PMF2_006.

-thalassemia trait in immigrants from Southeast Asia in Korea. *H. N. Kim[1], E. J. Lee[1], H. L. Kim[1,5], S. C. Jung[1], S. Y. Yi[2], E. A. Park[3], H. W. Chung[4]* 1) Dept Biochemistry; 2) Dept Internal Medicine; 3) Dept Pediatrics; 4) Dept Obstetrics and Gynecology, School of Medicine, Ewha Womans University, Seoul 158-710, Korea; 5) Korea National Institute of Health, Korea Centers for Disease Control, Seoul 122-701, Korea.

Beta-thalassemia is one of the most common hemoglobinopathy in many part of the world but rare in Korea. In the past 10 years, a dramatic increase in the number of immigrants from Southeast Asia into Korea led to have insight in the thalassemia. To detect -thalassemia trait, asymptomatic 635 Southeast Asian women immigrated to Korea were screened by using complete blood counts (CBC), Shine and Lal indices (MCV^2*MCH*0.01), Ricerca index (RDW/RBC) and peripheral blood smearing. Initially high risk group of -thalassemia trait was screened by two indices, either less than 79 fl mean corpuscular volume (MCV) or less than 26 pg mean corpuscular volume (MCH). Among 635 women, 72 were within the criteria. As a standard diagnostic marker for -thalassemia, the level of Hemoglobin (Hb) A_2 was measured by high performance liquid chromotography (HPLC). Among those 72 women, 13 women showed elevated Hb A_2 (> 3.5%), MCV 76 fl, MCH < 25 pg, Shine and Lal indices < 1530, Ricerca index < 4.4 and microcytic hypochromic red blood cell without anemia. These results implied they were -thalassemia tait. As immigrants from these countries increases, estimating allele frequencies of -thalassemia among them would be helpful to genetic counseling. This study was supported by the Center for Genome Science, Korea, National Institute of Health research contract (budgets: 2007-090-091-4854-300, 2008-E00157-00).

The genetic control of microRNA expression in Humans. *C. Borel, S. Deutsch, H. Attar, M. Gagnebin, C. Gehrig, E. Falconnet, Y. Dupré, S. E. Antonarakis* Dept Genetic Medicine, Univ Geneva Medical Sch, Geneva, Switzerland.

MicroRNAs (miRNAs) are small non-coding RNAs that regulate the expression of protein-coding transcripts. Each miRNA is thought to have multiple target genes that are regulated at the post-transcriptional level. Inter-individual variation of miRNA gene expression is likely to influence the levels of target transcripts, and may therefore contribute to some phenotypic differences, including susceptibility to common disorders. The aim of this study was to characterize the natural variation in miRNA expression levels present in different individuals, and to identify loci that control this variation. We established primary fibroblasts from 200 unrelated umbilical cord samples of Caucasian origin (GenCord collection). All of these samples have been genotyped using the Illumina Hap550 SNP array. After multiple filtering steps 417820 SNPs were retained for statistical analyses. Taqman real-time PCR in all cell lines was used to measure the expression of 365 known mature miRNAs in each sample. This revealed that 108 miRNAs are expressed in fibroblasts and that their expression levels are highly variable between individuals. Normalized miRNA levels for each individual were used to perform quantitative whole genome association studies using the Plink software. We will present the detailed genome-wide association analysis of SNPs that control in *cis*- or *trans*- miRNA expression. We identified around 5% of microRNAs with cis eQTLs and 16% with *trans* eQTLs that remain significant after multiple testing correction. *Cis* eQTLs are located quite far away from miRNA (200-700 kb) and a large fraction of *trans* eQTLs are intronic (13 out of 18). This is the first attempt to characterize the genetic regulation of miRNA expression levels. Loci identified through this approach are likely to be important determinants of human phenotypes. *C.B and S.D contributed equally to this work.*.

L-2-Hydroxyglutaric aciduria: clinical, genetic and neuroradiological findings in an Italian patient. *R. Mazzei[1], C. Ungaro[1,2], F. L. Conforti[1], T. Sprovieri[1], V. Blasi[1], A. Mollo[3], O. Gallo[1], P. L. Lanza[1]* 1) ISN-CNR, C.da Burga, Mangone (CS), Italy; 2) Department of Neurosciences, Psychiatric and Anesthesiological Sciences, University of Messina, Messina, Italy; 3) ASP 4, Cosenza, Italy.

In our report we describe clinical, genetic and neuroradiological features in an Italian patient with L-2-Hydroxyglutaric aciduria (L-2-HGA), in whom L-2-HGA was diagnosed at an adult age. L-2-HGA is an autosomal recessive inherited neurometabolic disease, belonging to the group of organic acidurias, whose biochemical hallmark is represented by increased levels of 2-hydroxyglutaric acid in urine, blood and cerobrospinal fluid, with 90% of isoforms representing the L-form. The gene causing L-2-HGA, designed as L2HGDH, encodes for a L-2-hydroxyglutarate dehydrogenase, a putative mitocondrial protein converting L-2-hydroxyglutarate to alpha-ketoglutarate. The mutations found in L-2-HGA patients abolish the activity of this enzyme, therefore the symptoms are most likely due to the toxic effects of the accumulating metabolite L-2-hydroxyglutarate; in fact, L-2-hydroxyglutarate has been shown to induce oxidative stress and to inhibit mitochondrial creatine kinase in the cerebellum. The proband underwent to the screening of the full L2HGDH gene by direct sequencing. Patients MRI scanning of the brain revealed hyperintense signal on T2W and FLAIR images of the subcortical white matter and basal ganglia. The symptoms were represented by mental deficiency, cerebellar ataxia, pyramidal and extrapyramidal signs and dysarthria. Molecular analysis showed a sequence variation in the exons 8, highlighting the presence of a homozygous nucleotide variation at position c.1082 (c.1082 CT) that leads to substitution of a CGA codon encoding for an arginine residue with a TGA codon predicting for a stop codon at position 335 (Arg335Stop) in the protein. In conclusion, in the current study we report the first evidence of a mutation in the L2HGDH gene in an Italian patient affected by L-2-HGA, reinforcing the previously described phenotype of this rare metabolic disease and reaffirming the data that mutations in the L-2-Hydroxyglutaric dehydrogenase gene cause L-2-Hydroxyglutaric aciduria.

Identification of the allelic architecture of *NNMT* gene as a genetic determinant for Homocysteine levels. *M. Sabater-Lleal[1], J. Martín[2], A. Buil[1], M. Chillón[3], JC. Souto[4], J. Blangero[5], J. Fontcuberta[4], F. Blanco-Vaca[2], JM. Soria[1], L. Almasy[5]* 1) Unit of Genomics of Complex Diseases, Hospital de la Santa Creu i Sant Pau (HSCSP), Barcelona; 2) Department of Biochemistry, HSCSP, Barcelona; 3) Institució Catalana de Recerca i Estudis Avançats (ICREA), and Centre de Biologia Animal i Teràpia Gènica. UAB. Barcelona; 4) Unity of Thrombosis and Hemostasia, HSCSP, Barcelona; 5) Southwest Foundation for Biomedical Research. San Antonio (TX).

Homocysteine (Hcy) plasma level is an independent risk marker for venous thrombosis, myocardial infarction, stroke, congestive heart failure, osteoporotic fractures and Alzheimer disease. Previous results from the GAIT Project allowed the identification of a region in chromosome 11q23, which was completely linked (Lod score =3.01;p=0.0001) Hcy levels. The most probable candidate gene in that region was the *NNMT* gene, a methyl-transferase involved in Hcy metabolism. A study of 17 SNPs in this gene reveled the presence of a haplotype strongly associated with Hcy levels (p=0.00031). To identify the most likely functional variants responsible for this association, we now have resequenced this candidate gene. We amplified the *NNMT* gene (75Kb) in two haplotype carriers. The fragments were cloned in TOPO XL vectors. We fully sequenced four clones of each individual and four clones from a control haplotype to obtain the complete sequence of the gene. The identified genetic variants have been analyzed in a sample of 398 GAIT participants using Bayesian QTN analysis. Sixty-six polymorphisms were identified in both carriers of the Hcy-associated haplotype. Preliminary multivariant results from the full GAIT sample show that four of these SNPs exhibit a strong probability of affecting Hcy levels (posterior probability >0.9). Using a novel strategy, we have identified four SNPs in the *NNMT* gene that present a strong probability of affecting Hcy levels. We are now analyzing the functional effect of these allelic variants. These results represent a relevant advance in the knowledge of the genetic factors that determine Hcy levels, an important intermediate phenotype for thromboembolic disease.

Population Genetics and Genomics of Human Gene Expression. *B. E. Stranger[1], S. B. Montgomery[1], C. E. Ingle[1], M. Sekowska[1], C. Beazley[1], C. Barnes[1], S. McCarroll[2], S. Tavaré[3,4], M. E. Hurles[1], P. Deloukas[1], E. T. Dermitzakis[1]* 1) Wellcome Trust Sanger Institute, Hinxton, UK; 2) Broad Institute of Harvard and Massachusetts Institute of Technology, Cambridge, MA; 3) Department of Oncology, University of Cambridge, Cancer Research UK, Cambridge Research Institute, Li Ka Shing Centre, Cambridge, UK; 4) Program in Molecular and Computational Biology, University of Southern California, CA.

Gene expression is a heritable, quantitative phenotype that can be used to assess the impact of naturally occurring functional genetic variation. It is increasingly used to assist in the interpretation of disease association signals. We previously described a comprehensive analysis of the genetic basis of gene expression variation in the original 270 individuals of the HapMap project using both SNPs and CNVs (Stranger et al. Science 2007 and Nat Genet 2007). We have now extended this analysis to 830 unrelated individuals from 8 global populations of the extended HapMap samples. We interrogated whole-genome gene expression in lymphoblastoid cell lines, and performed association analyses with these expression data and 1.6M SNP genotypes from the HapMap3 Consortium and thousands of high resolution CNV calls, with the goal of identifying and characterizing cis eQTLs in these populations. The increased power from analyzing nearly double the sample size of each of the initial four HapMap populations allows us to detect approximately 2.5 times the number of previously-reported genes with SNP-expression eQTLs in each of these populations, corresponding to a cis-eQTL for approximately 6% of expressed genes from samples of around 100 unrelated individuals. In total we detect cis-eQTLs for approximately 20% of expressed genes, with 34% of detected eQTL observed in at least two populations. We identify strong CNV-expression associations and observe that only 7-14% (depending on population) of CNV associations are also detectable with SNPs. We present analyses of population differentiation and natural selection on variants that influence gene expression using haplotype-based methodologies designed to detect recent selection.

Genome-wide association studies on multiple system atrophy (MSA). *Y. Nakahara[1], Y. Momose[2], Y. Ichikawa[1], Y. Takahashi[1], K. Yamamoto[3], J. Goto[1], S. Tsuji[1], JAMSAC (Japan Multiple System Atrophy research Consortium)* 1) Department of Neurology, Univ. of Tokyo, Tokyo, Japan; 2) Neurology, KITAHARA Nerurosurgical Institute, Tokyo, Japan; 3) Division of Molecular Population Genetics Department of Molecular Genetics Medical Institute of Bioregulation, Univ. of Kyushu, Fukuoka, Japan.

Background: Multiple system atrophy (MSA) is a sporadic neurodegenerative disorder characterized by various combinations of autonomic failure, cerebellar symptoms, parkinsonism and pyramidal signs. Although the discovery of alpha -synuclein has been identified as a major component of the glial cytoplasmic inclusions (GCIs), a pathologic hallmark for MSA, the etiologies of MSA remain to be elucidated. To obtain clues as to the genetic factors for MSA, we have conducted genome-wide association studies on MSA cases and controls. Design/Methods: We have established a consortium focusing on multiple system atrophy (JAMSAC; JApan MSA Research Consortium), to obtain longitudinal clinical information and genomic DNA. We genotyped 209 patients with MSA and 220 neurologically normal controls, using Illumina HumanHap 550K Genotyping BeadChip. Results: Genotype data (544,148 SNPs) fulfilling the following conditions are processed for statistical analyses; the call rate exceeding 0.98 in MSA patients and controls, p value for Hardy-Weinberg equilibrium exceeding 1E-6. The number of the SNPs with significant p values of 2 test are as follows; (p0.05: 24,024 SNPs, p0.01: 4,748 SNPs, p0.001: 482 SNPs, and p0.0001: 47 SNPs). To identify susceptibility genes for MSA further replication with independent data set will be required.

PALB2 and BRCA2 mutations in Finnish prostate cancer cases - Co-occurrence of mutations in a family with multiple types of cancer. *T. Wahlfors[1], S. Pakkanen[1], S. Siltanen[1], MP. Matikainen[2], TLJ. Tammela[2], J. Schleutker[1]* 1) Laboratory of Cancer Genetics, IMT, University of Tampere, Tampere, Finland; 2) Division of Urology, Tampere University Hospital and Medical School, University of Tampere, Tampere, Finland.

A truncating mutation (1592delT) in the recently found Fanconi anemia gene, PALB2 (BRCA2 cofactor), is associated with an increased breast cancer risk in Finland, but also may be correlated with an increased risk of other cancers including prostate cancer (PRCA). Besides breast cancer, mutations in the BRCA2 gene predispose carriers to PRCA in some population but PRCA-associated mutations in BRCA2 have not previously been identified in the Finnish PRCA patients. We explored the contribution of PALB2 and the BRCA2 PALB2-binding site mutations to the clinical characteristics of Finnish PRCA patients. Finnish families with two or more PRCA-affected males (178) and 285 unselected cases with complete clinical data were initially screened for mutations in the coding region and splice sites of PALB2. Additionally, we analyzed BRCA2 exon 2 and 3, which code for the PALB2 binding site, to address the implication of the BRCA2-PALB2 interaction on PRCA patient clinical parameters. We identified six variants in the PALB2 and five variants in the BRCA2 genes. No novel truncating mutations were found in either gene, but three novel variants were characterized in BRCA2. One family with multiple cancer cases, including four PRCA, two breast, one gastric and one skin, carried PALB2 1592delT, a BRCA2 missense mutation and two novel intronic BRCA2 variants. PALB2 1592delT showed complete penetrance in this family and clinical characteristics among the mutation carriers in this family revealed a trend towards aggressive disease. Aggressive fenotype associating with 1592delT also applied to a few non-familial cases. All together, our results indicate that, in Finland, PALB2 truncating mutations and potentially functional variants in BRCA2 exons 2 and 3 are rare at the population level. However, cancer incidence among mutation carriers is moderate to high and a trend towards aggressive disease is observed.

Novel Homozygous 4bp Deletion in LAMB2 Causing Pierson Syndrome. *M. Al-Owain[1], M. Faiyaz Al-Haque[2], H. Al-Zaidanl[1], H. Al-Muallimi[2], J. Nelson[3], H. Abalkhail[2]* 1) Dept Medical Genetics, King Faisal Specialist Hosp, Riyadh, Saudi Arabia; 2) Dept of Pathology and Molecular Medicine, King Faisal Specialist Hosp, Riyadh, Saudi Arabia; 3) Genetic Services of Western Australia, King Edward Memorial Hospital, Australaia.

Pierson Syndrome (Microcoria-Congenital Nephrosis Syndrome, MIM # 609049) has been recently delineated as an autosomal recessive disease causing congenital nephrotic syndrome and distinct eye abnormalities. The renal disease is classically due to diffuse mesangial sclerosis. The Microcoria (fixed narrowing of the pupils) is due to maldevelopement of the eye including hypoplasia of the iris, lens, and ciliary body. Pierson syndrome is caused by a defect in laminin beta 2, an integral component of basement membranes. We report two brothers who died in the infantile period with Pierson syndrome. During the pregnancy of the first affected child to consanguineous parents, the fetus was noted to have enlarged echogenic kidneys and markedly elevated amniotic fluid alpha-fetoprotein suggesting congenital nephrosis. After delivery, the newborn had massive proteinuria and had to undergo bilateral nephrectomy. The microscopic finding was consistent with diffuse mesangial sclerosis and diffuse dilatation of the renal tubules. The patient additionally had hypotonia, seizures, and microcephaly. Examination of the eyes revealed that the left eye was larger than the right and the pupils remained small despite administration of dilating drops with only glimpses of the fundi being apparent. The MRI of the brain showed areas of infarction in the parieto-occiptal lobe and the occipital lobe with dilatation of the lateral ventricles. The diagnosis of Pierson syndrome was made, however no mutation analysis of the gene was available. The family had subsequently another boy who was also affected with Pierson syndrome. LAMB2 gene sequencing revealed that he was homozygous for a novel 4 bp deletion in exon 26. Both parents were heterozygous carriers of this deletion. Based on this finding we have referred the family for preimplantation genetic diagnosis.

Identification of a mutation causing encasement of fetus. *J. Lahtela[1], H. O. Nousiainen[1], J. Tallila[1], H. Honkala[1], M. Gentile[2], V. Stefanovic[3], VM. Ulander[3], R. Karikoski[4], R. Salonen[5], M. Kestila[1]* 1) National Pub Hlth Institu, Helsinki, Finland; 2) Genome Informatics Unit, University of Helsinki, Helsinki, Finland; 3) Department of Obstetrics and Gynaecology, Helsinki University Hospital, Helsinki, Finland; 4) HUSLAB Central Pathology Laboratory, Helsinki University Hospital, Helsinki, Finland; 5) Department of Medical Genetics, Väestöliitto, Helsinki, Finland.

We describe a Finnish family with two affected fetuses with identical severe malformations. Both upper and lower limbs seemed to be absent because they were totally encased under the skin. The skull was small, and the nose and the mouth were replaced by a single orifice with a septum dividing it vertically into two spaces resembling open choanae. There were hypoplastic eyes on both sides of the orifice, and an abnormal large cyst in the middle of the neck. The pregnancies were terminated at 13+6 and 12+5 gestational weeks, respectively, based on ultrasound findings. Assuming recessive mode of inheritance, we performed a genome-wide scan with microsatellite markers using DNA samples of parents, affected fetuses and a healthy sibling. The linkage analysis provided several putative loci. We also performed genome-wide gene expression analysis from fibroblast RNA of affected fetuses and observed several differentially expressed transcripts compared to age-matched controls. Based on these findings and additional information from public databases, we sequenced a selected candidate gene from the affected fetuses. This study revealed a pathogenic mutation that creates a premature stop codon and co-segregates with the phenotype within the family. We are currently sequencing control samples to define the carrier frequency of the variant in the Finnish population. We are also performing functional studies to identify the actual consequences of the mutation on protein level. The findings of this study provide strong evidence about the role of this gene and protein in epidermal differentiation during human embryonic development and also add essential knowledge about the early fetal stages in general.

Segmental copy number variation shapes tissue transcriptomes. *A. Reymond[1], C. N. Henrichsen[1], N. Vinckenbosch[1], S. Zollner[2], E. Chaignat[1], S. Pradervand[1], M. Ruedi[3], H. Kaessmann[1]* 1) The Center for Integrative Genomics, University of Lausanne, Switzerland; 2) University of Michigan, Ann Arbor, MI; 3) Natural History Museum, Geneva, Switzerland.

Copy number variation (CNV) of DNA segments has recently been identified as a major source of genetic diversity, but a comprehensive understanding of the phenotypic effect of this type of variation is only beginning to emerge. We have generated an extensive map of CNV in wild mice and classical inbred strains. Copy number variable regions cover a total of ~340 megabases (~11%) of their autosomal genome. Genome-wide expression data from 6 major organs and 4 developmental times in six different strains reveal that expression levels of genes within CNVs positively or negatively correlate with copy number changes in approximatively 35 and 15% of the cases, respectively. Our experiments also show that CNVs influence the expression of genes in their vicinity - an effect that extends up to half a megabase. These controls over expression are effective throughout mouse development, however some genes appear to be under compensatory loops at specific time point. Interestingly, genes within CNVs show lower expression levels and more specific spatial expression patterns than genes mapping elsewhere in the genome. Furthermore, genes expressed in the brain are significantly underrepresented in CNVs compared to genes with expression in other tissues, suggesting differential selective constraint on copy number changes of genes expressed in different tissues. Our study provides initial evidence that CNVs shape tissue transcriptomes on a global scale and thus represent a significant source for within-species phenotypic variation.

Effect of enzyme replacement therapy on growth in patients with Hunter syndrome: results from the Hunter Outcome Survey. *M. Beck[1], J. Muenzer[2], M. Scarpa[3], G. Schulze Frenking[1], E. Wraith[4] on behalf of the HOS investigators* 1) Pediatric Dept, Univ Mainz, Mainz, Germany; 2) Pediatric Dept, Univ North Carolina, USA; 3) Pediatric Dept, Univ Padova, Padova, Italy; 4) Willink Biochemical Genetics Unit, Royal Manchester Children's Hosp, Manchester, UK.

Introduction: The Hunter Outcome Survey (HOS) is a global database covering the natural history of Hunter syndrome (MPS II) and the safety and effectiveness of enzyme replacement therapy (ERT) with idursulfase (ElapraseTM; Shire HGT). Untreated patients with MPS II have markedly reduced growth after 5-8y of age. The first data on the effects of ERT on the growth of patients with MPS II are provided here. Methods: On 15 May 2008, there were 421 prospective patients in HOS (i.e. alive when enrolled); 221 were receiving ERT. This analysis included all patients with available height data 1y before and 1y after ERT. Results: Height data were available for 29 patients; 2 were excluded because they were aged 20y at the start of ERT (baseline). Of the 27 eligible patients, 17 were aged 12y (3-11) and 10 were aged 12y (12-18) at baseline. Mean (SD) changes in height were: 2.9 (2.3) cm before and 4.7 (3.0) cm after ERT for patients aged 12y; 1.8 (2.3) cm before and 3.2 (2.7) cm after ERT for patients aged 12y; and 2.5 (2.3) cm before and 4.1 (2.9) cm after ERT for all patients. Mean (SD) change in height velocity 1y before to 1y after ERT was +1.6 (3.1) cm/y, and mean changes in z-scores were -0.5 (0.3) before and -0.1 (0.5) after ERT. Changes were more notable in patients aged 12y at baseline. Mean (SD) changes in height velocity for patients 12y and 12y were +1.8 (3.4) and +1.4 (2.5) cm/y, respectively. Mean changes in z-scores for patients 12y before and after ERT were -0.4 (0.2) and 0.0 (0.4), respectively, and for patients 12y were -0.5 (0.5) and -0.3 (0.5), respectively. Conclusions: In a non-age-stratified analysis of outcome survey data, we observed an acceleration of growth in patients with MPS II given ERT with idursulfase for 1y. Ongoing collection of data in HOS, including analysis of ERT and pubertal development status (Tanner stage), will further elucidate the effect of idursulfase ERT on growth.

Mechanisms of loss of heterozygosity in neurofibromatosis type 1-associated plexiform neurofibromas. *H. Kehrer-Sawatzki[1], K. Steinmann[1], L. Kluwe[2], R. Friedrich[2], D. N. Cooper[3], V.-F. Mautner[2]* 1) Human Genetics, Univ Ulm, Ulm, Germany; 2) Department of Maxillofacial Surgery, University Hospital Eppendorf, Hamburg, Germany; 3) Institute of Medical Genetics, School of Medicine, Cardiff University, Cardiff, UK.

Plexiform neurofibromas constitute a serious burden for patients with neurofibromatosis type 1 (NF1), a common autosomal dominant disorder characterised by pigmentary changes and tumorous skin lesions (neurofibromas). Despite the prominence of these benign tumours in NF1 patients, the mechanisms underlying the tumour-associated loss of heterozygosity (LOH) in plexiform neurofibromas have not been extensively studied. We performed LOH analysis on 43 plexiform neurofibromas from 31 NF1 patients, the largest study of its kind to date. A total of 14 (33%) plexiform neurofibromas exhibited LOH involving 17q markers. In four tumours, LOH was found to be confined to the NF1 gene region. However, in none of the tumours was a somatic NF1 microdeletion, mediated by non-allelic homologous recombination (NAHR) between either NF1-REPs or SUZ12 genes, detected. Thus, NF1 microdeletions do not appear to be frequent somatic events in plexiform neurofibromas. Determination of NF1 gene copy number by multiplex ligation-dependent probe amplification (MLPA) indicated that although tumours with smaller regions of LOH were characterized by 17q deletions, no NF1 gene copy number changes were detected in seven plexiform neurofibromas with more extensive LOH. This is the first indication that mitotic recombination is a frequent cause of LOH in plexiform neurofibromas.

Genes relating synapse in 7q31 as Candidate genes for Autism. *N. Nakashima, T. Yamagata, Z. Yu, M. Saito, M. Mori, M. Y. Momoi* Dept Pediatrics, Jichi Medical Univ, Tochigi, Japan.

Because genes working on synapse such as *NLGN3, 4, SHANK3* and *GABRB3* were identified as causative genes for Autism spectrum disorder (ASD), genes relating to synaptogenesis and function can be candidate genes. In linkage analysis, 7q31 was reported to be one of the locus for ASD. Therefore, we analyzed genes relating to synapse on 7q31, *NRCAM* and *GRM8*, and also *POT1* on ASD patients for mutation. *NRCAM* is expressed in neurons in the mature brain, and is considered to play a role in synapse formation and neuronal plasticity regulating cell-cell interactions. *NRCAM* was reported to associate with ASD. GRM8 is a member of metabotropic glutamate receptors that is a family of G-protein coupled receptors. *GRM8* is expressed in the brain especially cerebellum and fetal brain, and regulates glutamate transmission and maturation of synapse. *GRM8* was reported as an autism causative gene. POT1 is a conserved single-stranded DNA binding protein with the crucial function of the protection of telomeres and also the maintenance of overall genomic stability. *SHANK3* is located in the minimal telomeric region that rationalized *POT1* to be a candidate for ASD. (Methods)Genomic DNA was extracted from leukocytes/lymphoblasts from 63 Japanese patients obtained with the informed consent of their parents, and 100 Caucasian patients from AGRE (the Autism Genetic Resource Exchange). Each exon and introns nearby was amplified by PCR and sequenced. (Results) In *NRCAM*, we detected 22 sequence variations and 8 were in the coding region. One base change that induced amino acid change was detected in 20 patients and reported as SNP previously. Three SNPs were not reported previously. In *GRM8*, we detected 4 intronic base changes and one base substitution in the coding region that did not induce amino acid change. In *POT1*, we detected 18 sequence variations, and 5 of them were in the coding region. Three induced amino acid change, however, they were also detected in the controls. (Discussion) We did not detect causative mutation in *NRCAM*, *GRM8* and *POT1*. These results suggested that the causative genes should carry additional or much specified biological functions besides synaptogenesis.

Novel IRF6 mutations associated with nonsyndromic Cleft Lip or Cleft Palate: Who should be eligible for IRF6 screening? *K. M. Rocha[1], B. Burin[1], F. S. Jehee[1], L. Brito[1], P. S. Costa[1], R. Zechi-Ceide[2], N. Alonso[3], L. Dalva-Lopes[4], J. Souza[5], M. R. Passos-Bueno[1]* 1) Centro de Estudos do Genoma Humano, IB, USP; 2) Centro de Reabilitação, Bauru, USP; 3) Faculdade de Medicina, USP; 4) Defeitos da Face; 5) CAIF, PA, Brasil.

Nonsyndromic Cleft Lip with or without Cleft Palate (NSCLP), one of the most common human congenital malformations, is a multifactorial disorder. Recurrence risk varies from 4-10%. In contrast, Van der Woude syndrome (VWS), the most common syndromic form of CLP is an autosomal dominant condition with high penetrance caused by null mutations in IRF6. Lower-lip pits, present in about 85% of the VWS cases, allow the clinical distinction between VWS and NSCLP or NS Cleft Palate (CP). , Misdiagnosis of VWS can lead to incorrect recurrence risk calculation for the patients and their family. In order to test the proportion of NSCLP or NSCP with mutations in the IRF6, we have directed sequenced exons 3, 4 and 7 of IRF6 in 108 probands with CLP or CP (76 with CL/P, 24 CL, 6 CP and 2 congenital healed cleft lip) with at least one other affected relative with orofacial cleft. None of them had lower lip pits. Four missense mutations were detected: R9W, Q17P, V113L and D354N (c.1060G>A). These mutations were not detected in 200 control chromosomes and, except for the R9W mutation, were not previously described. Arg9, Gln17 and Val113 are located in evolutionary conserved amino acids at the DNA-binding domain and c.1060G is an evolutionary conserved guanine residue located 1 nucleotide upstream of the exon 7 donor site and they are probably pathogenic. We observed that three of these 4 mutations occurred in families segregating both CLP and CP. Therefore, these results are showing for the first time that CLP or CP in the absence of lower lip-pits can be associated with mutations in the IRF6 gene and we recommend that familial cases (1st degree affected relative), particularly those segregating CLP and CP, should be tested for IRF6 mutations CEPID/FAPESP, CNPq.

Genomic DNA duplications associated with complex polysyndactyly. *M. Sun[1], F. Ma[1], X. Zeng[2], Q. Liu[2], XL. Zhao[1], X. Zhang[1]* 1) Department of Medical Genetics, Peking Union Medical College, Beijing, China; 2) Peking Union Medical College Hospital, Beijing, China.

Triphalangeal thumb-polysyndactyly syndrome (TPTPS; OMIM 190605), syndactyly type IV (SD4; OMIM 186200) and tibial hemimelia-polysyndactyly-triphalangeal thumb syndrome (THPTTS; OMIM 188770) are congenital limb malformations all mapped to chromosome 7q36 and have various limb phenotypes including triphalangeal thumb, syndactyly and polydactyly. Unlike preaxial polydactyly types II (PPD2; OMIM 174500) and III (PPD3; OMIM 174600), which map to the same chromosome 7q36 region, no point mutation within the ZRS (ZPA regulatory sequence) was found in these complex polysyndactylies. We recently ascertained six Han Chinese families, including three with TPTPS, two with TPTPS and SD4, and one with SD4. We found that the pathogenic mutations underlying TPTPS/ SD4 were genomic DNA duplications involving ZRS. Using quantitative real-time PCR (qPCR) and Affymetrix-Genome-Wide Human SNP Array 6.0, we refined the extent of duplications, ranging from 144 kb to 398 kb. Sequence analysis of the breakpoints in three families suggested that the molecular mechanism underlying the genomic duplications might be non-homologous end joining (NHEJ). Our molecular findings suggest that severe syndactyly and complete thumb duplication seen in TPTPS and SD4 might be associated with unknown regulatory elements centromeric to the ZRS. The fact that two members in one of our TPTPS/SD4 families had limb phenotype similar to PPD2 also suggests that the ZRS duplication is probably responsible for those PPD in which point mutations were not discovered in ZRS. We predict that THPTTS could also be involved in the abnormal ZRS. Further genetic studies in more families may help to establish the correlation between the phenotypic severity and the extent of duplications.

Major loci determining lipid levels and coronary heart disease risk in 16 population-based European cohorts. *S. Ripatti[1,2], Y. Aulchenko[3], I. Lindqvist[1,2], CM. van Duijn[3], L. Peltonen[1,2,4,5] for the ENGAGE Consortium* 1) FIMM, Helsinki, Finland; 2) NPHI, Helsinki, Finland; 3) Dept of Epidemiology and Biostatistics, Erasmus University, Netherlands; 4) The Broad Institute, Massachusetts Institute of Technology, MA; 5) Wellcome Trust Sanger Institute, Hinxton, UK.

The recent flood of genome wide association (GWA) studies of lipid metabolism are based on heterogeneous samples of patients and controls derived from studies of diabetes and other disorders. Here we report the first GWA analysis of loci affecting lipid levels, major cardiovascular risk factors in 16 European population cohorts, not ascertained or collected based on any specific trait or disease outcome but sampled from the general population. Our study included a total of 17,798-22,562 persons (depending on the outcome studied), with age distributions ranging from 18 to 104 years and the geographic region of origin spanning from the Nordic countries to Southern European populations. We established 22 genomic regions consistently and significantly associated with serum lipid levels at a genome wide significance level (p5 x 10^{-8}), including 16 loci identified earlier by previous GWA studies. The six new loci with genome wide significance identified in our cohort samples are: ABCG5 (TC, p=1.5 x 10^{-11}; LDL, p=2.6 x 10^{-10}), TMEM57 (TC, p=5.4 x 10^{-10}), CTCF-PRMT8 region (HDL, p=8.3 x 10^{-16}), DNAH11 (LDL, p=6.1 x 10^{-9}), FADS3/FADS2 (TC, p=1.5 x 10^{-10}; LDL, p=4.4 x 10^{-13}) and MADD-FOLH1 region (HDL, p=6 x 10^{-11}). Jointly these 22 loci explain up to 5.6% of variation in lipids at the population level, the impact of some loci being different for males than females. When the impact of these loci was estimated for coronary heart disease risk in the Dutch cohort with individuals over 55 years of age, each risk allele increased the risk by 2%.

DNA methylation plasticity in the muscle tissue under disuse stress. *T. Kubota[1], K. Endoh[1], K. Ohori[1], K. Koyama[2]*
1) Fac Med, Epigenetic Medicine, Univ Yamanashi, Chuo, Yamanashi, Japan; 2) Fac Edu and Hum Sci, Univ Yamanashi,Kofu, Yamanashi, Japan.

The pattern of DNA methylation is faithfully maintained after DNA replication, and thus it has been thought that DNA methylation will not be changed for ones lifetime long. However, it has recently known that DNA methylation can be changed in short term by some factors such as malnutrition, mental stress, and some drugs as well as methylation supplements. To know the methylation plasticity, we investigate whether DNA methylation is changed by stress in rats muscle tissue. We examined DNA methylation in the upstream-regulatory regions of the three genes related to muscle differentiation in the soleus (lower limb) muscle tissues in acute-exercise (30 min on treadmill, 9 weeks of age) rat group (n=6, control=6), and those in chronic-disuse (3 weeks tail-suspension, 18 weeks of age) rat group (n=5, control=5) using bisulfite sequencing and COBRA methods. As a result, the percentages of methylated-CpGs in acute-exercise group were similar to the control group [57% and 42% in MyoD, 25% and 35% in Myogenin, 49% and 50% in Myostatin]. However, the percentages of methylated-CpGs in chronic-disuse group were different to the control group [60% and 27% in MyoD, 58% and 37% (24% and 17% (p=0.02) by COBRA) in Myogenin, 70% and 36% in Myostatin]. These results suggest that not acute-exercise but chronic-disuse stress changes DNA methylation in muscle tissue, and indicate that muscle tissue has DNA methylation plasticity. This observed hypomethylation in the muscles under the chronic-disuse stress may not only caused by proliferation of myoblast-like satellite cell induced by lower-limb muscle atrophy with tail-suspension (since these genes are hypomethylated in myoblasts), but also caused by demethylation change in the muscle cells in the atrophic muscle tissue without cell division (since the hypomethylation change is larger than the expected increase of the satellite cells).

Mutations in TMC1 are Relatively Frequent Among Families with Nonsyndromic Autosomal Recessive Deafness in Turkey. *M. Tekin[1], A. Sirmaci[1], H. Ozdag[2], D. Duman[1], H. Ozturkmen-Akay[3], S. Erbek[4], S. Tasir-Yilmaz[2], A. Incesulu[5], B. Ozturk-Hismi[1], Z. S. Arici[1], B. Yuksel-Konuk[1], F. B. Cengiz[1], I. Aslan[1]* 1) Division of Pediatric Molecular Pathology and Genetics, Ankara University School of Medicine, Ankara, Turkey; 2) Biotechnology Institute, Ankara University, Turkey; 3) Department of Radiodiagnostics, Dicle University School of Medicine, Diyarbakir, Turkey; 4) Department of Otorhinolaryngology, Baskent University School of Medicine Hospital, Konya, Turkey; 5) Department of Otorhinolaryngology, Eskisehir Osmangazi University School of Medicine, Eskisehir, Turkey.

Biallelic mutations in TMC1 have been reported to cause profound prelingual deafness, DFNB7/11. We screened TMC1 mutations in 86 unrelated Turkish families, in which 2-15 individuals with nonsyndromic severe to profound congenital or prelingual onset sensorineural hearing loss were born to normal hearing and consanguineous parents. GJB2 and mtDNA A1555G mutations were negative in probands from each family. Affected members in each family were initially genotyped for SNP or microsatellite markers linked with the TMC1 gene. Mutation analysis was performed in families showing co-segregation of deafness with haplotypes at the DFNB7/11 locus. A total of 6 different mutations in 7 families were identified, including previously undescribed three missense alterations, p.G444R (c.1330G>A), p.R445C (c.1333C>T), and p.I677T (c.2030T>C); one splice site mutation IVS6+2 T>A (c.64+2T>A); and a large deletion including exons 19-24; as well as a previously reported nonsense mutation, p.R34X (c.100C>T). All identified mutations co-segregated with deafness in all families and were not found in Turkish hearing controls. These results confirm the significant contribution of TMC1 mutations in deafness in Turkey.

Sign-dependent linkage disequilibrium estimation. *C. Zapata* Departamento de Genética, Centro de Investigaciones Biológicas Universidad de Santiago de Compostela, 15782 Santiago de Compostela, A Coruña, Spain.

Knowledge on the frequency and strength of non-random associations of alleles at different loci (LD, linkage disequilibrium) across genomes is very useful to explore their multilocus genetic architecture and to locate genetic factors underlying phenotype by positional cloning. In practice, most studies on LD follow estimation procedures which ignore the sign of deviations from random association. However, it is known that the sign of LD can affect seriously its estimation. The aim of the present work is to evaluate whether advantageous statistical properties to reliably estimate the frequency and intensity of LD between diallelic locus pairs concentrate on haplotype configurations exhibiting the same sign of LD. For this purpose, sign-dependent comparisons were performed by Monte Carlo simulation with respect to the power of the chi-square test to detect LD and the sampling variance and bias of estimates of the strength of LD obtained by the measure D'. The study included a wide variety of population conditions (different levels of LD intensity and combinations of allele frequencies at loci) and haplotype sample sizes. Sign-dependent LD analyses require the application of a uniform criterion to determine the allele composition of haplotype classes. Here, coupling haplotypes were designated as those carrying the most and the least frequent allele variants. Simulations showed that the power of the chi-square test was generally much higher for positive than for negative LD ($P < 0.01$). In addition, the precision of D'-estimates was also sign-dependent being the sampling variance generally much smaller for positive than for negative LD ($P < 0.01$). By contrast, no statistically significant differences in bias were found between D'-estimates with different sign over population conditions and sample sizes ($P= 0.32$). Overall, the present study shows that haplotype configurations with positive deviations from random association offer clear statistical advantages to reliably assess the frequency and intensity of LD across genomes with respect to sign-independent estimation procedures.

Folate Pathway Genes and Biochemical Studies in Orofacial Clefts. *S. Brustolin[1], M. E. Cooper[2], A. Silva[3], M. L. Marazita[2], M. M. Santos[1], R. Giugliani[1], J. C. Murray[3], T. M. Felix[1]* 1) Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil; 2) University of Pittsburgh, Pittsburgh,PA; 3) University of Iowa, Iowa City, IA.

Background: Non-syndromic cleft lip with or without cleft palate (CLP) is one of the most common birth defects with multiple genetic and environmental components. Studies have indicated that folate and vitamin consumption are environmental factors. Sample: Using 27 SNPs in 14 folate pathway genes, we analyzed 2 Brazilian samples consisting of 140 women (113 unaffected, 27 affected with CLP), their folate-related biochemical measures(vitamin B12, homocysteine, serum folate, red blood cell folate (RBC), hematocrit and hemoglobin) and 247 genotyped cleft proband trios where only some of the mothers have biochemical data. Results: NNMTrs2852447 shows significant differences in the means of the vitamin B12 among the 16 clefted mothers genotyped (ANOVA p=0.01) but not among all 140 mothers (ANOVA p=0.96). The means of all the biochemical measures do not differ by cleft status of the mothers (all t-test p>0.094). In chi-square tests of genotypes, the A allele of MTHFD1rs1950902 (p=0.01) and the CC genotype of CBSrs1789953 (p=0.01) are associated with clefted mothers. The TDT association tests on the trio family data revealed significant associations between clefting and: 1) The C alleles of MTRRrs1532268 (p=0.04), NNMTrs694539 (p=0.003) and BHMTrs651852 (p=0.04); 2) the CC genotypes of MTRRrs1532268 (p=0.02) and NNMTrs694539 (p=0.001); and 3) the haplotypes for the 4 SNPs in the BHMT gene (p=0.06) and the NNMT gene (p=0.03).The gene-gene interaction in each of these 3 SNPs with all the other SNPs was significant for MTRR rs1532268 versus 2 other SNPs within the same gene (rs10925235, p=0.03 and rs1801394, p=0.003) and NNMTrs694539 versus DHFRrs1643638 (P= <0.0001). Conclusion: The analysis indicates that the NNMT gene may be involved with both clefting and vitamin B12 and that both the MTRR and BHMT genes are involved with clefting. Further investigation with a larger sample will further illuminate the gene-environmental involvement with orofacial clefting. Grant NIH DE-08559, FIRCA TW007644-02 and CNPq.

The entire HFE gene deletion does not cause symptom of hemochromatosis in a homozygous woman of 47 years-old. *G. Le Gac, I. Gourlaouen, C. Ronsin, V. Géromel, A. Bourgarit, N. Parquet, S. Quemener, C. Le Maréchal, J.-M. Chen, C. Férec* Inserm U613, EFS-Bretagne, Université de Bretagne Occidentale, F-29200 France.

Hemochromatosis is predominantly associated with the HFE p.C282Y homozygous genotype, which is found in approximately 1 person in 200 in Northern European populations. Rarely, the disease also refers to point mutations in HJV, HAMP, TFR2 or SLC40A1 (namely the non-HFE hemochromatosis genes). Here, we identified an Alu element-mediated deletion of the entire HFE gene sequence, detected and further characterized using a range of PCR-based methods, and confirmed using array-comparative genomic hybrization (array-CGH). Although being homozygous for the HFE knocked-down allele, the woman did not present symptoms of hemochromatosis at 47 years-old. Our study illustrates the importance of including chromosomal rearrangement screening in the hemochromatosis diagnosis setting. But, it also supports the idea that not all individuals having a homozygous genetic alteration in HFE will be concern by clinical complications and especially the women.

Genotype and Phenotype Heterogeneity in MUTYH Associated Polyposis. *F. J. Hes[1], M. C. Joerink - van de Beld[1], M. Nielsen[1], N. Jones[2], S. Vogt[3], C. M. J. Tops[1], H. F. A. Vasen[4], S. Aretz[3], J. R. Sampson[2]* 1) Department of Clinical Genetics, Leiden University Meidcal Center, Leiden, Netherlands; 2) Institute of Medical Genetics, School of Medicine, Cardiff University, Wales, UK; 3) Institute of Human Genetics, University of Bonn, Germany; 4) Department of Gastroenterology & Medical Oncology, Leiden University Medical Center, Netherlands.

Background and aim: Bi-allelic mutations in the base excision DNA repair gene MUTYH lead to MUTYH associated polyposis (MAP) and thereby predispose to colorectal cancer (CRC). Functional studies have demonstrated significant differences in base recognition and glycosylase activity between various MUTYH mutations, notably for the two mutations most frequently reported in MAP patients: Y179C and G396D (previously annotated as Y165C and G382D). Our aim was to unravel correlations between genotype and their phenotypic expression of MAP. Methods: In this multicentre study we analyzed genotype and phenotype data including age at presentation of MAP, polyp count and the occurrence, location and age at presentation of CRC in 257 MAP patients. Results: The three possible bi-allelic combinations of truncating and non-truncating mutations showed no significant phenotypic differences. In contrast, both G396D homozygotes and G396D/Y179C compound heterozygotes presented later with MAP and had a significantly lower CRC hazard than Y165C homozygotes ($p<0,001$). The median age of developing CRC was 60 years and 51 years versus 48 years respectively. Conclusions: Our clinical study corroborated earlier functional assays and identified Y179C as a relatively severe and G396D as a relatively mild MUTYH mutation. Genotypic stratification may be useful in the development of guidelines for counseling, screening and management of families with MAP.

Dietary Intervention in Patients Receiving Substrate Reduction Therapy with Miglustat. *U. Ramaswami[1], H. Champion[1], J. Imrie[2], R. Lachmann[3], TM. CoX[4], JE. Wraith[2]* 1) Paediatric Metabolic Unit, Addenbrooke's University Hospital, Cambridge, United Kingdom; 2) Royal Manchester Childrens Hospital, UK; 3) Charles Dent Metabolic Unit, London, UK; 4) Department of Medicine, University of Cambridge, UK.

Background The iminosugar, miglustat, inhibits glucosylceramide synthase, the initial enzyme in the formation of glycosphingolipids: offering an alternative therapeutic approach in Type 1 Gaucher Disease and investigation into related lipidoses. Miglustat inhibits sucrase-isomaltase and other disaccharidases in the small intestine resulting in gastrointestinal (GI) side effects. Methods We analysed retrospectively the effect of dietary modification over a 6 month period in patients with related lipidoses. Patients were divided into Groups A, B, C as described in table below. Patients on low disaccharide diet commenced their modified diet 2-4 weeks prior to starting miglustat. Neurological outcome is not discussed for this presentation. Results 29 patients were included. Mean age 17 years(range 1-39). The results of mean change from base line in body weights are shown below. *

	Group A: Milk based; n=5	Group B: Low lactose; n=5	Group C Low Disaccharide; n=19
Mean Weight Change	-6.2	-1.7	+8.8
Mean Change in Weight in Line with Growth	-10	-4.3	+2.1

In Group A, frequent loose stools were controlled with loperamide; Group B had mild to moderate diarrhoea. Group C had occasional loose stools only. Conclusion Analysis of the data from 29 patients receiving therapy with miglustat shows that with dietary modification, weight gain during treatment can be maintained in line with weight gain potential and episodes of GI disturbances significantly reduced. A diet low in disaccharides was most effective especially when started before treatment commenced.

Transcriptome sequencing of human ESC. *G. Kolle[1], B. Gardiner[1], N. Cloonan[1], H. S. Chy[2], G. Q. Zhou[2], A. L. Laslett[2], S. M. Grimmond[1]* 1) Institute for Molecular Bioscience, University of Queensland, Brisbane, Qld, 4072, Australia; 2) Australian Stem Cell Centre, Clayton, Vic, 3800, Australia.

We have previously characterized the full polyadenylated transcriptome of mouse embryonic stem cells using Short Quantitative RNA Library (SQRL) sequencing (Cloonan et al., 2008)[1]. Here we present a comprehensive and complementary analysis of the transcriptome of human embryonic stem cells (hESC). To assess the full protein coding transcriptome, the polyadenylated fraction of hESC was isolated. To complement this, the membrane bound and free ribosome fractions of hESC were isolated using the method of (Diehn et al., 2000)[2]. This fractionation procedure permits a sensitive and accurate ability to distinguish all integral membrane and secreted proteins (extracellular space) that are translated by hESC. These three fractions were sequenced using the AB SOLiD next generation sequencing platform. A complete and quantitative estimation of gene expression level, spliced variant expression, novel transcription, non-coding RNA and transcribed sequence variants (SNPs, Indels) was performed. We were able to characterize all transcribed genes that encode integral membrane or secreted proteins, define spliced variants that result in switching from the membrane to the cytosolic fraction and reveal a set of novel genes not previously associated with the extracellular space in hESC. This is the highest resolution analysis of both the transcriptome of hESC and the membrane-polysome associated transcriptome of any mammalian cell type. References: 1. Cloonan N, Forrest AR, Kolle G, et al., Stem cell transcriptome profiling via massive-scale mRNA sequencing, 2008, Nat Methods, Epub ahead of print. 2. Diehn M, Eisen MB, Botstein D, Brown PO., Large-scale identification of secreted and membrane-associated gene products using DNA microarrays. 2000, Nat Genet 25: 58-62.

Indentification of genome wide targets of the UPF3B dependent nonsense-mediated mRNA surveillance pathway by exon array expression profiling and their relevance to the pathogenesis of intellectual disability. *J. Gecz[1], L. S. Nguyen[1], A. Gardner[1], L. Vandeleur[1], C. Shoubridge[1], J. Rodriguez[2], C.-F. Chen[3], L. Wang[2,3], A. Srivastava[2,3], M. Corbett[1]* 1) Dept Genetic Medicine, Women's & Children's Hospital, Adelaide, Australia; 2) J.C.Self Research Institute, Greenwood Genetic Center, Greenwood, South Carolina, USA; 3) Department of Genetics and Biochemistry, Clemson University, Clemson, South Carolina, USA.

Non-sense mediated mRNA decay (NMD) is a universal RNA surveillance pathway that also targets for degradation mRNAs bearing premature termination codons (PTC). We showed that mutations in UPF3B cause different forms of learning disability (Nature Genet, 39:1127-33, 2007). To assess the impact of UPF3B null mutations we performed expression profiling of control and patient lymphoblastoid cells using Human Exon 1.0 ST arrays. We found 633 deregulated genes (30% up, 70% down). One of these genes was UPF3B itself, which carriers PTC mutations in our patients. Applying KEGG pathway analysis we found disruption in regulation of cytoskeleton (PAK4, RHOA, FGFR4) and axon guidance (NFATC4, CFL2, NGEF), as well as purine and pyrimidine metabolism in the patients. Our data also demonstrated, that NMD is only partially compromised in the absence of UPF3B. Surprisingly, comparison with previous studies from UPF1, UPF2 or UPF3B knock down models in human, fly and yeast generated minimal overlap. Such low correspondence might be explained by tissue specificity of NMD, array platforms used or complexities of in vitro knockdown models versus naturally occurring human UPF3B KO situation. We also identified consequences of UPF3B KO on the level and type of alternative splicing and mRNA processing. At FDR=0.01 ($p<0.0006$), 1047 genes made the list. We manually picked and analysed 133 genes, which fell into two categories: 3 processing (34 genes, 25%; LMO2, XRN2, RPS8 and GARNL4); and internal splicing (100 genes, 75%; EIF4E3, UBAP2, PAPOLA and TBP). In summary, we have identified for the first time the bona fide targets of the UPF3B dependent NMD pathway and glimpsed into the complexities of NMD pathways and the molecular pathogenesis of UPF3B associated learning disability.

Linkage and Candidate Gene Studies of Autism in EU Populations. *R. Holt[1], G. Barnby[1], E. Maestrini[2], E. Bacchelli[2], D. Brocklebank[1], A. J. Bailey[3], A. P. Monaco[1], EU Autism MOLGEN Consortium* 1) The Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, UK; 2) Dipartimento di Biologia, Università di Bologna, Italy; 3) University Department of Psychiatry, Park Hospital for Children, Oxford, UK.

Autism is a strongly genetic childhood onset psychiatric disorder; however, genes underlying its susceptibility remain elusive. As part of an EU collaboration, we used linkage and haplotype analyses to refine previously identified regions of linkage with autism. We also examined variants in candidate genes for association with autism. The Autism Genome Project (AGP) have genotyped families, including 311 from the EU, using the Affymetrix 10k array. A total of 384 SNPs from this array within regions of linkage with autism on chromosomes 2, 3, 6, 7, 16 and 17 were genotyped using the Illumina GoldenGate BeadArray platform on 92 new EU multiplex families. The data from these new genotypes were pooled with those of the 311 EU families from the AGP study and used to perform a meta-analysis of linkage and to search for parent-of-origin effects. Also, 281 trios were genotyped to narrow critical regions by searching for extended haplotypes. For the candidate gene study, seven genes previously reported to be involved in autism, *ASMT*, *SHANK3*, *GRIK2*, *SLC6A4*, *NOSTRIN*, *PRKCB1* and *RELN*, were investigated. A total of 384 SNPs were selected to cover all variation in the genes. Genotyping was performed with the Illumina BeadArray platform on one proband and both parents from 387 multiplex families. Association was sought using the TDT. The highest LOD score in the linkage analysis was 1.64 (rs2862479, chromosome 3), increasing to 1.87 (rs2885116, chromosome 2) when a subset of Finnish families were excluded. Parent-of-origin and haplotype analyses are ongoing. In the candidate gene study, the most significant result was for rs362780 (*RELN*, $P = 0.00079$), although this does not reach significance after correction for multiple testing. While continuing to demonstrate the difficulties in identifying autism susceptibility loci, these results help highlight the possible importance of chromosomes 2 and 3, and *RELN* in autism susceptibility.

Breast cancer risk not only was not associated with CYP17/ A2 allele but also was related to A1 allele. *M. Hosseini[1], M. Houshmand[2,3], A. Ebrahimi[2,3]* 1) Dept Science, Islamshahr Branch,Islamic Azad University, Tehran,Iran, Asia; 2) National Institute for Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran; 3) Special Medical Center, Tehran, Iran.

Breast cancer is the second common cancer in rate in world and the first common cancer in Iranian women. The Cytochrome P-450c17 (CYP17) gene, located on chromosome 10q24.3, encodes the enzyme Cytochrome P-450c17, which functions as susceptibility factor in breast cancer. Three common polymorphisms have been described in the CYP17 gene [1, 2]. The variant creates a recognition site for the MspAI restriction enzyme, the common allele as A1 and the variant alleles A2 (-34TC). Totally 53 Iranian sporadic breast cancer affected women compare to control group were studied by PCR-RFLP for CYP17 variant. Even the A2/A2 were reported as risk factors for breast cancer our results showed that the A1/A1 were most risk factor in our population. A2/A2 had inhibition effect in our patients. [A1/A1 / A2/A2 odds ratio, 5.57 (95% confidence interval, 1.514-20.506) p=0.008]. We conclude that not only A2/A2 in our patients was not associated with breast cancer risk but also there is a reverse relation between presence of A1/A1 and increasing of breast cancer risk. Keywords: CYP17 gene, polymorphism, breast cancer, MSPA1, PCR-RFLP, susceptibility factor.

***Vax2* regulates retinoic acid distribution and cone opsin expression in the mouse eye.** *G. Alfano[1], T. Caramico[1], P. Dollè[2], S. Banfi[1]* 1) TIGEM, Fondazione Telethon, Rome, Rome, Italy; 2) Institut de Genetique et de Biologie Moleculaire et Cellulaire, Illkirch, France.

Vax2 is a homeobox gene expressed in the ventral developing eye. The inactivation of this gene in mouse determines an incompletely penetrant eye coloboma as well as abnormal axonal projections of ventral retinal ganglion cells to the superior colliculus. In order to dissect the molecular pathways in which *Vax2* is involved, we performed a transcriptome analysis of *Vax2-/-* mice starting from early stages of eye development until adult stages. By microarray studies, we found that some genes encoding enzymes involved in the metabolism of retinoic acid (RA) show significant variations of their expression levels in *Vax2-/-* mice. RA plays an important role at early stages of eye development and displays a peculiar distribution in the developing eye, as it is present only in its dorsal and ventral parts while it is absent in an intermediate region (RA-free zone). We found, in *Vax2-/-* mutants, an expansion of the expression domain of the genes encoding the RA-degrading enzymes *Cyp26a1* and *Cyp26c1* and a down-regulation of the RA-synthesizing enzyme *Raldh3*. This leads to an abnormal distribution of RA in the developing eye of *Vax2-/-* mice with a significant expansion of the RA-free zone in the ventral part of the eye. In addition, this analysis allowed us to detect, in *Vax2-/-* mice, an altered expression of two cone-specific genes, *S-Opsin* and *M-Opsin*, which plays a key role in color vision. These two genes normally display an asymmetric dorsal-ventral distribution in the eye. In our mutant animals, we observed a significant alteration of the expression gradient of these two genes, which may underline impairment in visual function. These data demonstrate that *Vax2* plays a key role in the determination of the distribution of RA in the developing eye and in the establishment of a physiological asymmetric expression of genes not only at early stages of development but also in adult stages and in mature photoreceptors. We propose that the expression alterations determined by *Vax2* in cone photoreceptors are mediated by the abnormal distribution of RA at earlier stages of eye development.

Analysis of candidate SNPs for high Hb F in thalassemia intermedia patients. *F. Anni[1,2], L. Perseu[3], S. Satta[1], M. Bowser[4], P. Fortina[2,5], M. Devoto[4,5], S. Surrey[6], R. Galanello[1]* 1) Ospedale Regionale Microcitemie, ASL Cagliari, II Clinica Pediatrica, Universita` degli Studi di Cagliari Cagliari, Italy; 2) Dept of Cancer Biology, Thomas Jefferson University, Philadelphia, PA; 3) INN-CNR, Cagliari, Italy; 4) The Children's Hospital of Philadelphia, University of Pennsylvania School of Medicine, Philadelphia, PA; 5) Dept of Experimental Medicine, University La Sapienza, Rome, Italy; 6) Cardeza Foundation for Hematological Research, Thomas Jefferson University, Philadelphia, PA.

Raising fetal globin levels in adult life could eliminate the need for transfusion in thalassemia and decrease painful crises in sickle cell disease. Since the population from the island of Sardinia is genetically homogeneous, identification of high Hb F determinants might be more straightforward compared to other populations. In this study, 43 transfusion-dependent (TD) and 29 non-transfusion dependent (NTD) or thalassemia intermedia individuals all homozygous for the 39 C->T chain termination mutation inherited on I, II or IX -globin haplotype backgrounds were evaluated for SNPs associated with the high Hb F phenotype. All were Xmn I negative at -158 G and no additional promoter variants or indel were detected in -like globin gene clusters. Initial focus was on sequence analysis of SNPs previously shown to be associated with high Hb F on chromosomes 2 and 6. rs6732518 located in the BCL11A gene on 2p16.1 showed highly significant association, with a frequency of the C allele of 0.52 in the NTD group and of 0.20 in the TD group ($p = 0.00006$). Less significant association was detected for rs1320963 on 6q23.3 ($p = 0.02$). Whole-genome association studies are ongoing using the Affymetrix GeneChip 6.0 array to elucidate other high Hb F determinants in this unique population.

Clinical and molecular investigation in an unusual Rett Syndrome case. *T. Sprovieri[1], R. Mazzei[1], C. Ungaro[1,2], A. Fiumara[3], A. Magariello[1], A. Patitucci[1], L. Citrigno[1,2], A. Arena[3], A. L. Gabriele[1], M. Muglia[1], F. L. Conforti[1]* 1) ISN/CNR, Mangone(CS), Italy; 2) Department of Neurosciences, Psychiatric and Anesthesiological Sciences, University of Messina, Messina, Italy; 3) Department of Paediatrics University of Catania, Catania, Italy.

Here we report the clinical and X-linked cyclin-dependent kinase-like 5 (CDKL5) molecular investigation in a very unusual Rett Syndrome (RTT) case, with severe, early-neurological involvement. Mutations in the CDKL5 gene have recently been reported in patients with severe neurodevelopmental disorder characterized by early-onset seizures, infantile spasms, severe psychomotor impairment and very recently, in Rett syndrome-like phenotype. Although the involvement of CDKL5 in specific biological pathways and its neurodevelopmental role have not been completely elucidated, CDKL5 appears to be physiologically related to the MECP2 gene. We investigated a 13-year-old girl affected by early convulsion onset who fulfilled the revised criteria for RTT variant phenotypes. Samples were also taken from more than 150 controls, matched for geographical region. DNA extraction and molecular investigation were performed by standard protocols. The screening of the whole coding sequence of the CDKL5 gene by DHPLC revealed an abnormal chromatographic pattern in exon 12 of the patient. Direct sequencing of the amplicon identified a CDKL5 missense mutation AACACC; c.1417 AC (GenBank accession No. Y15057), causing the aminoacidic change of asparagine in threonine (p.N399T). This sequence variation was not found both in the patients family and in more than 300 normal chromosomes. In conclusion, in our study we have identified a novel CDKL5 pathogenic mutation N399T that contributes to enlarge the CDKL5 gene variation database. Nonetheless, this finding reinforces the idea that altered CDKL5-regulation of MECP2 is responsible for a specific RTT phenotype and suggests that CDKL5 mutation screening should be performed in RTT patients, mainly females, presenting a history of early onset of a severe intractable seizure disorder.

Genome-wide association and meta-analysis for bipolar disorder in European ancestry samples. *L. J. Scott[1], P. Muglia[2], W. Guan[1], R. Upmanyu[3], M. Flickenger[1], X. Kong[4], F. Tozzi[2], J. Li[5], M. Burmeister[5, 6], D. Absher[7], R. C. Thompson[6], F. Meng[6], A. Farmer[8], J. Vincent[9], A. D. Roses[10], R. M. Myers[7], D. Burns[2], M. Boehnke[1] for GSK R&D and the Pritzker Neuropsychiatric Disorders Research Consortium & Site Directors* 1) Biostatistics, U Michigan, Ann Arbor, MI; 2) Genetics, GSK R & D, Verona, Italy; 3) GSK, Harlow, UK; 4) GSK, Research Triangle Park, NC; 5) Human Genetics, U Michigan, Ann Arbor, MI; 6) MBNI, U Michigan, Ann Arbor, MI; 7) HudsonAlpha Inst Biotechnology, Huntsville, Alabama; 8) IoP, King's Coll London, London, UK; 9) Psychiatry, U Toronto, ON, Canada; 10) Duke U Med Ctr, Durham, NC.

Bipolar disorder (BD) is a disabling and often life-threatening disorder that affects ~1% of the population worldwide. To identify genetic variants that increase the risk of BD, we genotyped 2 sample sets on the Illumina 550K Beadchip: (1) NIMH/Pritzker: 1195 BD 1 cases and 777 controls collected through the NIMH Genetic Initiative and the Prechter Repository and (2) GSK: 899 BD cases and 904 controls from London, Toronto, and Scotland. We tested for SNP-BD association in each sample and performed meta-analysis across samples. The NIMH/Pritzker-GSK meta-analysis yielded a top association p-value of 2.4×10^{-6}. We next imputed and analyzed data using the publicly available Affymetrix 500K genotype data from the Wellcome Trust Case Control Consortium (WTCCC) for 1,868 BD cases and 12,813 controls comprised of individuals from the National Blood Sample and from six non-BD case groups. After elimination of overlap of 261 cases present in both the WTCCC and GSK data, we performed a 3-study meta-analysis of 3,926 cases and 14,594 controls genotyped or imputed on 485,604 SNPs. A non-synonymous SNP on chromosome 3 showed the strongest 3-study meta-analysis association (p-value = 2.4×10^{-8}) and was associated in the NIMH/Pritzker, GSK, and WTCCC studies with p-values of .003, .001, and .00005, respectively. Genotyping and analyzing additional samples will be required to confirm that variant(s) in this region increase BD risk.

Comprehensive resequencing of *ABCD1, ABCD2, ABCD3* and *ABCD4* genes in patients with X-linked adrenoleukodystrophy (ALD) and association studies with the phenotypes of ALD. *T. Matsukawa[1], M. Asheuer[2], Y. Takahashi[1], J. Goto[1], Y. Suzuki[3], N. Shimozawa[3], H. Takano[4], O. Onodera[4], M. Nishizawa[4], P. Aubourg[2], S. Tsuji[1]*
1) Dept Neurology, Univ Tokyo, Tokyo, Japan; 2) UMR Inserm 745, University Paris-Descartes, Hôpital Saint-Vincent de Paul, Paris, France; 3) Univ Gifu, Gifu, Japan; 4) Univ Niigata, Niigata, Japan.

Background: Adrenoleukodystrophy (ALD) is an X-linked disorder affecting primarily the white matter of the central nervous system occasionally accompanied with adrenal insufficiency. Despite the discovery of the causative gene, *ABCD1*, clear genotype-phenotype correlations have not been established. Association studies based on SNPs identified by comprehensive resequencing of gene related to *ABCD1* may reveal genes modifying the phenotypes of ALD. **Methods:** We analyzed 40 Japanese patients with ALD (childhood cerebral CCALD 16, adult with cerebral AdultCer ALD 6, AMN with cerebral AMN-Cer ALD 2, AMN 13, asymptomatic 1 and Unknown 2). *ABCD1* and *ABCD2* were analyzed by newly developed microarray-based resequencing system. *ABCD3* and *ABCD4* were analyzed by direct nucleotide sequence analysis. Replication studies were conducted on an independent french ALD cohort with extreme phenotypes (CCALD 118 and pure AMN 71) **Result:** All the mutations of *ABCD1* were identified and there was no correlation with the ALD phenotypes. SNPs identified by comprehensive resequencing of *ABCD2*, *ABCD3* and *ABCD4* were used for association studies. There were no significant associations between these SNPs and ALD phenotypes, except for the 5 SNPs of *ABCD4* that are in complete disequilibrium in the Japanese population. These 5 SNPs were significantly less frequently represented in the patients with AMN compared to controls in the Japanese population ($p<0.05$), while there were no significant differences in patients with CCALD. The replication study employing these 5 SNPs on an independent french ALD cohort, however, did not show any significant associations with CCALD or pure AMN. **Conclusion:** The present study indicates that *ABCD2*, *ABCD3* and *ABCD4*, are less likely the disease modifying genes, necessitating further studies to identify genes modifying the phenotypes of ALD.

Haplotypic investigation of SNPs at the NOS2A locus in relapsing-remitting Multiple Sclerosis. *I. Manna[1], M. Liguori[1], P. Valentino[2], F. Condino[1], R. Nisticò[2], P. Spadafora[1], G. Di Palma[1], A. Quattrone[1,2]* 1) Institute of Neurological Science - CNR, Cosenza, Italy; 2) Institute of Neurology, University "Magna Graecia", Catanzaro, Italy.

The aim of this study is to investigate the contribution of the gene encoding the inducible form of Nitric Oxide Synthase (NOS2A) in Relapsing-Remitting Multiple Sclerosis (RRMS). This study enrolled 250 selected RRMS patients from Southern Italy and 250 ethnically matched healthy subjects. We decided to approach the NOS2A gene study with the SNPlex methodology, an efficient and reliable tool for a broad range of genotyping applications which uses oligonucleotide ligation, polymerase chain reaction and capillary electrophoresis to analyze bi-allelic single nucleotide polymorphism (SNP) genotypes. Thirteen SNPs spanning throughout the entire NOS2A gene have been selected. Genotyping was performed by the SNPlex platform and data was analyzed by GeneMapper v4.0 (Applied Biosystems), using the SPSS 12.0 software for the statistical evaluation. Genomic DNA was extracted from the peripheral blood samples of all the subjects recruited for the study, and the NOS2A genotypes of 56 RRMS patients and 40 controls have been analized. The distribution analysis of the SNPs frequencies showed that two of them (rs1137933 and rs2297518) seemed to be more frequently associated to the MS disease ($p= 0.0053$ and $p= 0.046$, respectively). Although derived from a limited sample of the study population, these data seem to confirm that NOS2A gene might be involved in the MS susceptibility; the evaluation of the remaining samples is in progress, also to eventually identify a disease-related NOS2A haplotype. Acknowledgement: The Authors thank dr. Letizia Gerace (Applied Biosystems Europe) for the technical support in the SNPlex methodology.

AMPD1 knock-out mice: A murine model for myoadenylate deaminase deficiency. *T. Morisaki[1,2], J. Cheng[1], N. Sugimoto[1,2], A. Dohi[1,2], E. Kimura[1], T. Shintani[1,2], M. Ikawa[3], M. Okabe[3], H. Morisaki[1]* 1) Dept Bioscience, Natl Cardiovasc Ctr Res Inst, Suita, Osaka, Japan; 2) Dept Molecular Pathophysiology, Osaka University Graduate School of Pharmaceutical Sciences, Suita, Osaka, Japan; 3) Genome Information Research Center, Osaka University.

AMP deaminase (AMPD)is an enzyme playing an important role in purine nucleotide interconversion in eukaryotic cells. AMPD1 gene, a member of the AMPD gene family in vertebrates, is specifically expressed in skeletal muscles, and its deficiency was reported in patients with metabolic myopathy, though it is controversial whether AMPD deficiency is indeed associated with exercise-induced muscle dysfunction. Also, it was reported that AMPD1 mutant allele have an improved clinical outcome of congestive heart failure but the mechanism for that has not been verified. To answer those questions, we generated a murine model for myoadenylate deaminase deficiency by establishing AMPD1 knock-out (KO) mice. Homozygous AMPD1 KO mice showed almost complete loss of AMPD activities only in skeletal muscles, though there were no abnormal histological findings in skeletal muscles. Physiological levels of exercise did not apparently alter the nucleotide levels in skeletal muscles nor showed different muscle performance in AMPD1 KO mice, while the electrostimulation of isolated muscle revealed elevated AMP and decreased ATP in muscle of those mice. Moreover, more phosphorylated AMP activated protein kinase (AMPK) was determined after electrostimulation in muscle of AMPD1 KO mice. In this study, we established a murine model of myoadenylate deaminase deficiency, and confirmed altered nucleotide metabolism and changed AMPK phosphorylation status in AMPD1 deficiency. Further functional analysis of this model is being in progress.

Hybrid SNPing provides a rapid technology to identify chromosome abnormalities, including balanced translocations, and genome-wide characterization of a species genome in the context of a different species genome. *R. D. Nicholls[1,2], S. Li[1], D. Lewis[2], S. M. Gollin[2], D. N. Finegold[1,2]* 1) Children's Hospital of Pittsburgh, Pittsburgh, PA; 2) University of Pittsburgh, Pittsburgh, PA.

We developed a genome-wide technology using single nucleotide polymorphism (SNP) genotyping microarrays and DNA from somatic cell hybrids, termed hybrid SNPing. This process allows the rapid, specific and complete characterization of the genome of one species in the context of another species genome, illustrated using two applications on a set of rodent-human somatic cell hybrids retaining one or more human chromosomes and chromosome fragments. First, hybrid SNPing provides a complete human genome molecular karyotype of somatic hybrid cells, to 1-50 kb resolution, which we verified by PCR and FISH methodologies. Second, hybrid SNPing provides high-resolution definition of chromosomal rearrangements such as balanced translocations associated with birth defects and other congenital disease. The average resolution of chromosome fragment and translocation breakpoint mapping from hybrid SNPing using Illumina 300K SNP genotyping arrays was 25.1 kb, with a range of 1.47 kb to 51.3 kb for the nine breakpoints that we characterized. Use of Illumina 550K SNP arrays resolved reciprocal balanced translocation breakpoints to 4.2 kb and 4.7 kb. To date, breakpoints of three balanced translocations [t(15;17), t(14;18, t(3;14)] were directly cloned by PCR and DNA sequencing. With 300K arrays, a maximum of 3% of human SNP probes are potentially conserved in rodents, which decreases to 1.5% with a more stringent cutoff and phylogenetic sequence analyses. In conclusion, hybrid SNPing identifies to near nucleotide resolution the DNA content and breakpoints of balanced translocations and chromosome fragments, with envisaged applications in somatic cell genetics to identify oncogenes, tumor suppressor genes, cell senescence genes, stem cell markers, or any functional sequence that can be screened for by cellular phenotypic assays. Other novel applications of the technology include identification of disease-associated SNPs and large DNA fragments in transgenic or gene therapy applications.

Syntrophin-2 that interacts with neuroligins is not related with autism. *M. Saito, T. Yamagata, N. Nakashima, M. Mori, M. Y. Momoi* Pediatrics, Jichi Medical University, Shimotsuke, Tochigi, Japan.

Neuroligin (NL) 3 and 4 were identified as responsible genes for autism spectrum disorder (ASD). NLs are cell adhesion molecule which are essential components of synaptogenesis, and the binding of NLs to -neurexin induces both pre- and postsynaptic maturation. Addition to that, NLs were reported to interact with syntrophin-2 (SNTG2), and the mutations of NLs detected in the autistic patients were reported to impair interaction between NLs and SNTG2. The syntrophin family is considered as adapter proteins that interact with dystrophin and the dystrophin-related proteins in both neuromuscular junctions and the CNS. ASD is more common in Duchenne and Becker muscular dystrophy than in the general population. From these points, we analyzed coding regions of SNTG2 for possible direct link between SNTG2 and ASD. Materials and Methods: Genomic DNA was extracted from the peripheral blood leukocytes/lymphoblasts from 143 ASD patients. Patients included 65 Japanese patients obtained with the informed consent of their parents, and 78 Caucasian patients from AGRE (the Autism Genetic Resource Exchange). Each exon and introns nearby were amplified by PCR and the PCR products were sequenced. Detected sequence variations were compared with control samples. Results: We detected 32 kinds of sequence variants and 12 of them were in the coding region. Ten base substitutions induced amino acid change and 4 of them were already reported as SNPs. The other 6 base changes were detected in 1 to 4 patients each, but also in the controls. Therefore, they were considered as rare SNPs. No association was detected in each SNPs between ASD and controls. Discussion: We did not detect any mutations or ASD-associated SNPs in STNG2. Our result suggested that SNTG2 was not one of the major causative genes for ASD.

Role of Optineurin Sequence Variations in the Ghana Patients with Primary Open-Angle Glaucoma. *MA. Hauser[1], Y. Liu[1], CS. Cohen[2], K. LaRocque-Abramson[1], X. Qin[1], C. Santiago-Turla[2], LW. Herndon[2], P. Challa[2], D. Budenz[3], S. Schmidt[1], S. Akafo[4], RR. Allingham[1,2]* 1) Ctr Human Genetics, Duke Univ Medical Ctr, Durham, NC; 2) Department of Ophthalmology, Duke University Eye Center, Duke University Medical Center, Durham, NC; 3) Bascom Palmer Eye Institute, Miami, FL; 4) Unit of Ophthalmology, Department of Surgery, University of Ghana Medical School, Korle Bu, Ghana.

Glaucoma is a heterogeneous group of disorders that cause retinal ganglion cell loss resulting in optic nerve degeneration. Glaucoma is the leading cause for irreversible blindness worldwide. Primary open-angle glaucoma (POAG) is the most common type and is highly prevalent in persons of West African descent. Optineurin (OPTN, GLC1E) was initially identified as the causative gene in a set of pedigrees displaying autosomal dominant normal-tension glaucoma (NTG). Subsequently, variants of this gene have been shown to be associated with POAG in Indian, German, and Japanese populations, while playing a much less significant role in US Caucasians. In this study, we have explored the role of OPTN in a population collected in the western African country Ghana. Entry criteria for this study included glaucomatous optic nerve damage, associated visual field loss, and elevated intraocular pressure (>22 mm Hg in both eyes). Unaffected controls were also collected. All exons of the OPTN gene were sequenced in 190 Ghanaian POAG patients and 140 controls. Several coding variants were identified, including the E322K variant (rs523747) in exon 10, which is significantly associated with POAG (Chi-square $p = 0.04$). There were no significant differences on the frequencies of other identified variants between POAG cases and controls in this population. Our study represents the first OPTN screening with POAG patients from western Africa and suggests that variants in OPTN may play a role in POAG from those of West African descent.

A Functional *MMP12* Promoter Polymorphism is Associated with Increased Lung Function and Delayed Onset of COPD. *G. Hunninghake[1], M. Cho[1], M. Soto-Quiros[2], L. Avila[2], J. Lasky-Su[1,3], C. Lange[1,3], D. Demeo[1], C. Hersh[1], B. Klanderman[1], B. Raby[1], D. Sparrow[4], S. Shapiro[5], E. Silverman[1], A. Litonjua[1], S. Weiss[1], J. Celedón[1]* 1) Channing Lab, Brigham and Women's Hospital, Boston, MA; 2) Hospital Nacional de Niños, San José, Costa Rica; 3) Department of Biostatistics, Harvard School of Public Health, Boston, MA; 4) VA Healthcare System and Department of Medicine, Boston University School of Medicine, Boston, MA; 5) Department of Medicine, University of Pittsburgh, Pittsburgh, PA.

Background: Genetic variants that influence lung function in children might ultimately contribute to the development of chronic obstructive pulmonary disease (COPD) in adults. Methods: We tested for association between single nucleotide polymorphisms (SNPs) in the gene for matrix metalloproteinase 12 (MMP12) and a measure of lung function (pre-bronchodilator FEV1) in over 5,400 subjects in five cohorts in: two family-based studies of childhood asthma, and three populations of adults including populations of individuals and families recruited on the basis of a subject with COPD, and a prospective study of initially healthy men in the Normative Aging Study (NAS). Within NAS, we also tested for association between SNPs associated with FEV1 and time to onset of COPD, and replicated findings in adults with severe, early-onset COPD. Results: Two correlated SNPs (rs737693 and rs2276109 [a known functional promoter variant]) were significantly associated with pre-bronchodilator FEV1, in populations of both children and adults, and in a combined analysis of all cohorts (P values=7.0 x 10-5 and 5.0 x 10-4, respectively). These SNPs were associated with a significantly reduced risk of COPD in subjects from the NAS (e.g., hazard ratio for rs2276109= 0.65, 95% confidence interval=0.46-0.92, P=0.02) and explained 28% of the population attributable risk of COPD. Similar associations with COPD were noted in a family-based study of subjects with early-onset COPD (P = 0.04 rs2276109). Conclusions: SNPs in MMP12 are associated with a reduced risk of COPD in adulthood, likely through a positive effect on FEV1 level in early life.

Transmission of KID syndrome by a mosaic parent for a *GJB2* mutation. *M. Titeux[1], V. Mendonça[3], A. Décha[1], E. Moreira[3], S. Magina[3,4], A. Maia[3], L. Lacaze-Buzy[2,5], J. E. Mejía[1], L. Torrão[3,4], F. Carvalho[4], J. Eça-Guimarães[3,4], A. Hovnanian[1,2,5]* 1) INSERM U563, Toulouse, France; 2) Reference Centre for Rare Skin Diseases, Toulouse, France; 3) Hospital de São João do Porto, Porto, Portugal; 4) University of Porto School of Medicine, Porto, Portugal; 5) Purpan Hospital, Department of Medical Genetics, Toulouse, F-31000 France.

KID syndrome associates keratitis, ichthyosiform lesions and neurosensory deafness. This is an autosomal, dominant disorder caused by specific, often sporadic mutations in the genes encoding connexin-26 and connexin-30 of gap junctions (*GJB2*, *GJB6*). Over 80% of known cases are caused by the recurrent c.148G>A (p.Asp50Asn) mutation of *GJB2*. We describe the first instance of KID syndrome transmission from a mosaic parent. The proband was a 27-month-old Portuguese boy born to non-consanguineous parents. He developed generalized keratotic plaques of the skin more prominent on the scalp and face, had no hair, eyebrows and eyelashes, showed dystrophic nails and palmoplantar hyperkeratosis. Deafness was clinically detected, and he developed bilateral corneal opacification. Cerebellar hypoplasia was observed by magnetic resonance imaging, in association with neuromuscular defects. The father was healthy, while the mother displayed hyperkeratotic, pigmented, segmental lesions on the chest and back along the Blaschko lines. Leukocyte DNA showed heterozygosity for the p.Asp50Asn mutation of *GJB2* in the patient but not in his parents. The mutation, however, was weakly detectable in the mothers lesional skin DNA, suggesting mosaicism, and its presence was confirmed by molecular cloning of the PCR amplimers. KID syndrome is thus a member of a growing group of disorders which can present with segmental cutaneous manifestations arising from mosaicism. It argues for systematic careful clinical assessment of parents of KID patients, and has consequences for genetic counseling due to the risk for their offspring of inheriting a generalized form of the disorder.

The association between genetic polymorphism of INSIG2 and obesity in Central Taiwan. *K.-Y. Tung[1], R.-Y. Wang[2], K.-T. Chen[1], C.-J. Lin[3], M.-C. Hung[3], W.-C. Shy[3], C.-Y. Chen[2], T.-N. Wu[1], F.-Y. Wu[1]* 1) China Medical University, Environmental Health, Taichung, Taiwan; 2) China Medical University, Public Health, Taichung, Taiwan; 3) China Medical University, Nursing, Taichung, Taiwan.

In recently, diet change on food increasing rate of obesity prevalence in the world. The lipid metabolic gene, insulin-induced gene 2 (INSIG2), is putatively involved in the transcription of genes associated with regulating the lipid metabolism, and may play a key role in lipid metabolism. Our study aims are to investigate putative associations between genetic polymorphism of INSIG2 and obesity related indices. A total of 691 subjects participate our study. Subjects were recruited in 2007 from Central-western; Hsin-yi village in Nantou country (240 aboriginal subjects) and Taichung city (447 non-aboriginal subjects) in Taiwan. All subjects were adult recipients of national health insurance screening examination. Data on demographics, lifestyle habits and medical history were collected using a structural questionnaire. The subjects with a BMI 27 were classified as obese (n = 213). The rs7566605 polymorphism in the INSIG2 gene was genotyped by TaqMan assay. SAS statistical software was used to analysis the distribution of INSIG2 genotypes between the obese and non-obese groups. The results demonstrated that aborigines had a higher BMI than non-aborigines (28.5 kg/m2 vs. 24.3 kg/m2, p 0.0001). The subjects that carried GG genotype of INSIG2 had a higher BMI than GC or CC genotypes (26.3 kg/m2 vs. 25.5 kg/m2, 25.2 kg/m2; p = 0.03). After adjusting for confounding factors such as age, gender, ethnicity and waist circumference, the odds ratio for obesity that carriers the GC or CC genotype compared to the GG genotype was 0.55 and 0.33 (95% CI: 0.34 - 0.9 ; 95% CI: 0.15 - 0.7), respectively. In conclusion, our study showed that the GG genotype of rs7566605 on INSIG2 may as a risk factor for obesity. These findings may provide potential preventions or therapeutic strategies.

Relationship between the thrombophilic mutations or single nucleotide polymorphisms and pulmonary thromboembolism. *F. Atac[1], H. Verdi[1], AC. Yazici[2], A. Simsek[3], F. Eyupoglu[3], N. Ozbek[4]* 1) Dept Medical Biol & Genetics, Baskent Univ Fac Medicine, Ankara, Turkey; 2) Dept Biostatistcs,Baskent Univ Fac Medicine, Ankara, Turkey; 3) Dept Chest Disease and Tuberculosis, Baskent Univ Fac Medicine, Ankara, Turkey; 4) Dept Pediatric Heamotology, Baskent Univ Fac Medicine, Ankara, Turkey.

Thrombosis, is a complex and multifactorial disease that results from the interaction between predisposing of both inherited and acquired risk factors. There has been considerable interest in identifying molecular risk factors that predispose to thrombosis. The disequilibrium in haemostatic system is the key mechanism that enrolles at types of thrombosis. The point mutations and/or polymorphisms in the genes encoding coagulation factors shifts the delicate balance in haemostatic system toward thrombosis. Several genetic risk factors, especially factor V Leiden and prothrombin G20210A mutations have been reported to be related to VTE in Caucasians, but the relationship remains controversial in other populations. Since the prevalence of molecular risk factors for thrombosis varies greatly in different parts of the world, both in patients with thrombosis and in the general population, we felt it was prudent to evaluate further the relation between the thrombophilic mutations / single nucleotide polymorphisms and pulmonary thromboembolism phenotype in our population. 95 patients that are diagnosed as pulmonary thromboembolism by thorax CT or ventilation-perfusion scan were included in our study. Restriction fragment size analysis were performed by visualizing digested PCR products for Factor V Leiden (FVL G1691A), Factor V Cambridge (A1090G), Factor V A1299G, prothrombin G20210A, methylene tetrahydropholate reductase C677T . and Plasminogen activator inhibitor 1 4G/5G. The results of this study indicate that FVL (0.001) , Prt G20210A (0.001) and MTHFR C677T (0.001) may be suggested as risk factors for pulmonary thromboembolism in our population.

MATERNALLY DERIVED CHROMOSOME 1 IN A SIBLING PAIR WITH MENTAL RETARDATION, MICROCEPHALY, HYPOTONIA, EPILEPSY, FACIAL DYSMORPHISM, ATAXIA AND IMPAIRED SPEECH: CLINICAL AND MOLECULAR FINDINGS. *D. Aktas[1], GE. Utine[1], A. Aydin[1], K. Mrasek[2], A. Weise[2], N. Akarsu[1], T. Liehr[2], M. Alikasifoglu[1], E. Tuncbilek[1]* 1) Dept Genetics, Hacettepe Univ, Ankara, Turkey; 2) Universitatsklinikum Jena, Institut für Humangenetik und Anthropologie, Jena, Germany.

Whole-genome analysis using SNP oligonucleotide arrays allows identification of microdeletions, duplications and uniparental disorders. Here, we report on a sibling pair with mental retardation, microcephaly, hypotonia, epilepsy, facial dysmorphism, ataxia and impaired speech. GTG-banded chromosome analysis revealed as 46,XX,der(1)(:p34.2-q43~44::p34.2->pter). FISH and MCB analysis showed that the deletions were at 1p34.2 and 1q44. SNP oligonucleotide array analysis also detected these deletions as 2.9 Mb in size at 1p34.2 and 2.7 Mb in size at 1q44. The deleted region on 1p34.2 encompasses 33 genes, among them is GLUT1 gene (SLC2A1) (MIM*606777). Haploinsufficiency of GLUT1 leads to GLUT1 deficieny syndrome, which is characterised by epilepsy, developmental delay, microcephaly, hypotonia, ataxia and impaired speech. GLUT1 gene deficiency seemed to be most relevant in relation to phenotype of our patients. On the other hand, deleted region on 1q44 contains zinc finger protein and olfactory receptor genes. Since zinc finger genes have previously been shown to be involved in mental retardation, these zinc finger genes (SMYD3, ZNF695, ZNF670, ZNF669, ZNF124, ZNF496 and ZNF672) which are located at 1q44 may be related with mental retardation in our patients. The clinical phenotype of our individuals have clear similarities with previous cases, suggesting a specific phenotype of patients with 1q deletions. During the parental evaluation, the paternal karyotype was found normal. However, the maternal karyotype with GTG-banding as 46,XX,t(1;1)(p34.2;q44),del(6)(q16.1q21). To the best of our knowledge, this is the first report of a phenotypically normal woman with an interstitial deletion of region 6q16.1q21. With SNP array, breakpoint-associated imbalance on chromosome 1 was not identified; however, a deletion at 6q16.1q21 15.9 Mb in size was detected in the mother.

Changes in global DNA methylation in response to chronic consumption and withdrawal of folic acid is dependent on the MTHFR 677CT polymorphism. *K. Crider[1], E. Quinlivan[2], R. J. Berry[1], L. Hao[3], Z. Li[3], D. Maneval[2], T. Yang[2], S. Rasmussen[1], Q. Yang[1], J. Zhu[2,3], L. Bailey[2]* 1) NCBDDD, Division of Birth Defects and Developmental Disabilities, CDC, Atlanta, GA; 2) University of Florida, Gainesville, Fl; 3) Peking University Health Science Center, Beijing, China.

We evaluated the effect of the MTHFR CT polymorphism on global DNA methylation from a double-blind randomized trial in which Chinese women of reproductive age took supplements of folic acid (100, 400, 4000 g/day). DNA methylation was expressed as a % of methylated cytosines (measured by a novel LC-MS/MS assay) at enrollment, 1, 3, 6 months of supplementation, and after a 3 month washout period, stratified by MTHFR genotype (CC, CT, TT) (n=135; ~15 subjects/genotype x 3 treatment groups). Baseline methylation levels (4.4 0.12) were similar (p>0.25) across the MTFHR genotypes. Folic acid supplementation resulted in a 13.5% (p< 0.0001) decrease in global methylation after 1 month of exposure, independently of genotype and dose. After 6 months of supplementation, DNA methylation had returned to baseline in the TT-subjects taking 100 g, but not in CC-subjects, or in any subject receiving 4 mg/d. During the 3 month washout period DNA methylation decreased further in CC (40%) and CT (20%), but not significantly in the TT-subjects. This post-intervention decrease in methylation was both genotype and dose dependent. In conclusion, global DNA methylation response to folic acid supplementation and withdrawal varied by MTHFR genotype with a complex genotype-dose interaction.

New preimplantation genetic diagnosis for fatal surfactant deficiency caused by ABCA3 mutations. *B. Tazon-Vega, C. Zhang, K. Amoroso, Z. Rosenwaks, KP. Xu* CRMI, Weill Cornell Medical College, New York, NY.

Mutations in the ABCA3 gene can lead to lethal conditions caused by a deficiency in pulmonary surfactant. Our objective was to provide PGD to a couple with two specific ABCA3 mutations (intron 6: 613+4 a>g and exon 10: 1132g>a) together with linkage markers that would reduce the risk of misdiagnosis due to allele dropout (ADO) and could be applicable to other patients in the future. The couple had an affected neonate who died of surfactant deficiency. DNA was extracted from paraffin embedded tissue obtained from the deceased neonate. Four microsatellite markers flanking the ABCA3 gene were selected defining a 0.8 Mb region: D16S3024 - 0.68 Mb - D16S291 - 0.01 Mb - ABCA3 - 0.02 Mb - D16S664 - 0.09 Mb - D16S663. To detect the parental mutations, two restriction enzyme reactions were set up (HphI for intron 6 and AcuI for exon 10). Linkage and mutation analysis were performed for the family to determine haplotypes linked to the ABCA3 mutations. Markers and mutations were co-amplified in a pentaplex PCR from single lymphocytes to validate the method. Overall amplification efficiency was 83.4% and markers showed 10.3% of ADO. In this family markers D16S3024, D16S291 and D16S663 indicated the paternal and maternal carrier and normal alleles. For marker D16S291 the couple did not share any of the possible alleles allowing assessment of ADO. Marker D16S664 was not informative. Two IVF-PGD cycles were performed. Overall 17 eggs were retrieved, 15 were fertilized and all were biopsied obtaining 16 blastomeres. Two embryos were diagnosed as unaffected, 5 carriers (maternal) and 6 affected. Inconclusive results were obtained in two embryos showing the maternal normal haplotype and absence of the paternal haplotype. One recombination was detected between markers D16S3024 and D16S291. Unaffected embryos were not transferable due to poor development. Two embryos were transferred in both cycles resulting in a biochemical pregnancy the first time and no pregnancy the second time. One carrier embryo was cryopreserved. A new indication for molecular PGD is now available to reliably detect ABCA3 mutations causing fatal surfactant deficiency.

CA/TG-repeat instability as a factor impeding branch migration in Holliday junctions during homologous recombination. *M. G. Yakubovskaya[1], V. K. Gasanova[1], N. V. Ryadninskaya[1], G. A. Belitsky[1], V. I. Popenko[2], C. Gaillard[3], F. Struass[3]* 1) Institute of Carcinogenesis, Blokhin Cancer Rsearch Center RAMS, Moscow, Russia; 2) Engelgardt Institute of Molecular Biology, RAS, Vavilova 32, Moscow 117984, Russia; 3) Centre de Recherche des Cordeliers, 15 rue de l'Ecole de Medecine, 75006 Paris, France.

Homologous recombination provides an error-free way for double-strand break repair. However, its role in carcinogenesis of inherited cancer via loss of heterozygosity was demonstrated to exceed impacts of point mutagenesis. Homologous recombination can be modulated by a variety of factors, and deep knowledge of its molecular background is essential to control this type of genetic rearrangements. Some microsatellites represent hot-spots of homologous recombination. CA/TG-repeats, the most abundant microsatellites in the human genome, affect homologous recombination. One of the possible mechanisms is by the formation of noncanonical DNA structures impeding branch migration in Holliday junctions. We have studied this hypothesis with Holliday junctions formed by two PCR products, which sequences were identical except for an internal region containing either a random sequence or a (CA)31-repeat. A comparative electron microscopy analysis of the Holliday Junctions revealed a statistically larger number of cross points in the internal regions of structures with repeats. Internal CA-regions of different duplexes were shown not to interact. Using native polyacrylamide gel electrophoresis in combination with restriction fragment length polymorphism analysis, one-side or one-round amplifications and electrophoresis in denaturing conditions, we showed CA-repeat instability in PCR causing formation of homo- and heteroduplexes. As CA-repeat instability greatly exceeds DNA-polymerase error both in vitro and in vivo, and since microsatellite heterozygosity by several repeat units is widely spread in eukaryotes, this repetitive sequence heterology appears to be a structural background for formation of Holliday junctions with repetitive sequences at the cross-points, that enhances probability of homologous recombination termination in the repeat region.

Further Explorations in the Genetics of Age-related Maculopathy (ARM). *D. E. Weeks[1,2], J. Jakobsdottir[2], Y. P. Conley[1,3], R. E. Ferrell[1], M. B. Gorin[4]* 1) Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, PA, USA; 2) Department of Biostatistics, Graduate School of Public Health, University of Pittsburgh, PA, USA; 3) Department of Health Promotion and Development, School of Nursing, University of Pittsburgh, PA, USA; 4) Department of Ophthalmology and Jules Stein Eye Institute, The David Geffen School of Medicine, UCLA, CA, USA.

A combination of strategies have been used to successfully identify susceptibility genes for ARM including family-based linkage studies, locus-specific and genome-wide SNP, and candidate-based genotyping. We have integrated the results of multiple linkage studies and the AREDS public genome-wide association findings with an extensive set of 587 genes based on known and hypothesized biological pathways in the etiology of ARM. We created a prioritization strategy to develop a panel of 1381 SNPs for association studies with the Center for Inherited Disease Research. A gene was selected if 1) AREDS associated P-value 0.005, 2) AREDS associated P-value 0.05 and ranked among top 50 genes based on HLOD or S_{all} ranks from our local linkage scan, 3) AREDS associated P-value 0.05 and located in a region supported by the meta-analysis of ARM linkage scans, 4) ranked among top 50 genes based on local linkage scan and located in a region supported by at least one other scan, 5) AREDS associated P-value 0.05 and located in a region supported by at least one linkage scan other than ours, and 6) located in a region supported by at least two linkage scans other than ours. This left 111 genes of interest, 73 of those are known to be expressed in the eye. SNPs were tested for allelic associations using the MQLS statistic. We successfully replicated known associations for SNPs linked to CFH, C2, C3, and HTRA1/LOC387715 with P-values 10^{-28}, 10^{-6}, 10^{-5}, and 10^{-12}, respectively. We also found several complement factor H related loci and a few novel genes that do not appear to be directly related to the complement activation pathway. We are pursuing replication studies of these new loci in a separate cohort.

The diploid genome sequence of an Asian individual. *J. Wang* Beijing Genomics Institute at Shenzhen, Shenzhen, 518083, Huada Genomics Institute, Shenzhen University Medical School, Shenzhen, 518083, China.

We present the diploid genome sequence of the first Asian individual. The genome was sequenced to 36-fold average coverage, using massive parallel sequencing technology. By aligning the short reads onto the NCBI reference genome, 99.6% of the genome has been covered, and variations, detected using the assembled high-quality consensus sequence, covered 92% of the whole genome. Approximately 3 million SNPs were identified, of which 13.6% were not in dbSNP. SNP detection accuracy was measured by comparing the assembly with the genotyping analysis and showed that 99.2% of the 1M genotyped alleles have been covered at a 99.9% consistency. The majority of the remaining unconfirmed SNPs were validated using PCR amplification and Sanger sequencing. The high amount of consistency in the alleles of the assembly with those identified via genotyping indicates that this sequence is the highest quality of all currently available human genome sequences. The heterozygotes were phased, and haplotypes were predicted against HapMap CHB/JPT haplotypes. Paired-end reads were used to discover structural variations and short indels. We carried out further analysis on identified variations that might potentially change gene function or be associated with known diseases or phenotypes. Our data and characterization of the variations demonstrated the usefulness of next-generation sequencing technologies for personal genomics.

Polymorphisms of plasminogen activator inhibitor 1 gene associated with diffuse type gastric cancer susceptibility. *H. Ju, C. Kang* Biological Sciences, KAIST, Daejeon, Korea.

Plasminogen activator inhibitor 1 (PAI1) regulates physiological thrombotic and fibrinolytic processes by inhibiting plasminogen activator. It also has roles in regulating tumor invasion, angiogenesis and metastasis, and many clinical studies have found that high levels of PAI1 are correlated with poor prognosis for cancers. In this case-control association study using 250 Korean gastric-cancer patients and 400 non-patient controls, we found that several single-nucleotide polymorphisms (SNPs) in PAI1 were significantly associated with susceptibility to diffuse-type gastric cancer. The most significant association was found for a SNP in the intron 7 region and the risk variant increased the susceptibility to diffuse-type gastric cancer by 1.4-fold (P = 0.0019) versus the non-risk variant, but not the susceptibility to intestinal-type gastric cancer. When fused to the luciferase gene, the intron 7 sequence reduced the luciferase activity by 4-fold. No significant difference was found, however, between the risk and non-risk variants. Further studies are in progress towards identifying functional polymorphisms affecting the susceptibility to diffuse-type gastric cancer.

Analysis of IRF6 gene in Van der Woude syndrome from an Indian population. *A. Ali[1], S. Singh[2], R. Raman[1]* 1) Department of Zoology, Banaras Hindu University, Varanasi, India; 2) G S Memorial Hospital, Varanasi, India.

One of the most common congenital malformations, the cleft lip and/or cleft palate (CL/P) when associated with lower lip pits is known as Van der Woude syndrome (VWS), the most common form of CL/P. Till now 2 loci have been mapped for VWS; 1q32-41 and 1p34. Only one gene IRF6 which resides in 1q32-41 region is known to cause VWS in various populations. Mutations were found in 70% of VWS cases. More than 70 mutations have been reported to date in IRF6 in VWS cases. There is no report of genetic study of VWS on Indian population. To screen for mutations in IRF6 gene in VWS cases from an Indian population, resequencing of all coding regions and exon-intron boundaries of IRF6 gene in 13 individuals (9 affected and 4 unaffected) from 7 Van der Woude syndrome families as well as 5 normal controls was performed. We have detected 5 novel variants; IVS1+ 3900 A>G, 191 T>C, IVS4+ 775 C>T, IVS8+218 C>T, 1511 T>A (Ser 416 Arg) and 2 known variants; IVS6+27 C>G, 1083G>A (V274I). Except one, all were in non-coding regions either in 3 UTR or in introns, while one was in coding region detected in a normal control. In this cohort, we report lack of any mutation in the coding region of the affected individuals. All but one of the variants obtained in probands were also found in one or other unaffected family members and/or controls. In an independent case-control study from this population, on the association of IRF6 820G>A SNP with nonsyndromic cleft lip with or without cleft palate (NSCLP) on 761 individuals including probands, their parents and normal controls, we have found the frequency of putative risk allele G in the probands (0.88) to be higher than the normal controls (0.81) but lower than mothers (0.89), and fathers (0.92). This study showed a statistically significant difference of IRF6 820G allele between the case and controls but this appears to be only a weak risk factor for NSCLP in this population. Though screening a larger number of VWS samples will allow a more reliable view, the present report indicates that point mutations in IRF6 gene may not be a major cause of VWS in Indian population from the eastern region.

Broad clinical spectrum in Silver-Russell syndrome and consequences for genetic testing. *N. Schoenherr[1], S. Spengler[1], D. Gonzalez[2], M. Arslan-Kirchner[2], G. Binder[3], T. Eggermann[1]* 1) RWTH Aachen; 2) MH Hannover; 3) University Tübingen.

Silver-Russell syndrome (SRS) is a heterogenous disorder characterised by severe intrauterine growth restriction, lack of catch-up after birth (IUGR/PNGR) and specific dysmorphisms. In ~10% of patients maternal uniparental disomy of chromosome 7 (UPD(7)mat) is detectable. Hypomethylation of the ICR1 in 11p15 can be discovered in ~63% of typical SRS patients fulfilling strict criteria defined by Netchine et al. (JCEM 2007:92:3148-54) (length/weight at birth -2SD; 3/5 of the following criteria: PNGR, relative macrocephaly, asymmetry, prominent forehead, feeding difficulties). Single patients carry chromosomal aberrations. Due to the heterogeneity of SRS the clinical diagnosis is difficult and the detection rate for disturbances is relatively low. We tested 161 SRS patients from routine diagnostics and 20 patients referred as isolated IUGR/PNGR for 11p15 (epi)mutation and UPD(7)mat. In the SRS group ICR1 hypomethylation was identified in 23 and UPD(7)mat in 4 patients. None of the patients with isolated IUGR had a 11p15 (epi)mutation but two carried a UPD(7)mat. Interestingly some SRS patients with a ICR1 hypomethylation showed only slight IUGR (>-2SD) while the two isolated IUGR/PNGR patients with UPD(7)mat were retrospectively identified as mild SRS. Despite the scarce clinical data we can show that the phenotypic spectrum is broad and that several (epi)mutation or UPD(7)mat carriers would be missed if we consider strict diagnostic criteria. Based on our experience we propose to test (a) severely growth retarded patients (-2SD) even when only minimal SRS features are present and (b) probands with isolated asymmetry and mild growth retardation. Testing should include: (1) Methylation-specific (MS) MLPA for detection of 11p15 (epi)mutations. (2) MS-PCR for the PEG1/MEST locus. In case of UPD(7)mat the results should be confirmed by microsatellite typing to exclude (so far unreported) isolated imprinting defects. Although the MS-PCR test is restricted to a narrow region in 7q it would detect all reported segmental UPD(7)mat cases. (3) Cytogenetic analyses should be done.

Description of a four-generation family with autosomal dominant cerebellar ataxia: clinical and genetic analysis.

F. Annesi[1], D. Italiano[2], G. Provenzano[1,3], P. Tarantino[1,3], E. V. De Marco[1], D. Civitelli[1], A. Laganà[3], A. Gambardella[1,4], P. Bramanti[2], G. Annesi[1], A. Quattrone[1,4] 1) Inst Neurological Sci, National Research Council, Mangone, Cosenza, Italy; 2) IRCCS Centro Neurolesi Bonino-Pulejo, Messina, Italy; 3) Department of Neurosciences, of Psychiatric and Anaesthesiological Sciences, First Neurology Clinic, University of Messina, Italy; 4) Institute of Neurology, University Magna Graecia, Catanzaro, Italy.

SCAs are a heterogeneous group of neurodegenerative disorders, principally characterized by imbalance, dysarthria. To date, about 29 genetic loci associated to Mendelian forms of SCA are known, and a number of SCA genes have been identified. We performed a genetic analysis of a family from southern Italy, with a form of slowly progressive SCA. 14 subjects (7 affected) from a SCA family with 15 affected members in 4 generations have been evaluated. The disease seems to display an autosomal dominant pattern of inheritance with elevated penetrance. The mean age at onset was 34 years with a strong evidence of anticipation across generations. The first symptoms were invariably legs heaviness, imbalance, or dysartria. We performed mutational analysis by PCR searching for the most common SCA mutations. Two-point linkage analysis for known SCA genetic loci was calculated with the LINKAGE program package. Mutational analysis excluded pathological repeat expansions in the SCA 1, 2, 3, 6, 7, 8, 12, 17 and DRPLA genes. Linkage exclusion tests showed no evidence of association with most of known mapped SCA loci (SCA4, SCA5, SCA13-16, SCA19-22, SCA25 and SCA27-29). SCA prevalence in Italy significantly differs from European countries because of the very low prevalence of SCA 3 and SCA 6. 45% of the Italian families are associated with SCA1 and SCA2 genotypes, and only a small percentage of cases exhibited expansions in SCA3, SCA6, SCA7, SCA17 and DRPLA genes. In this large family, mutational analysis excluded the presence of all the main common SCA gene mutations, and linkage analysis ruled out the association with most of the so far reported SCA genetic loci. This could suggests a genetically distinct form of SCA.

Characteristics of SNPs Associated with Complex Traits in Genome-Wide Association (GWA) Studies: The NHGRI GWA Catalog. *L. A. Hindorff, J. P. Struewing, E. M. Ramos, H. A. Junkins, T. A. Manolio* Office of Population Genomics, NHGRI, NIH, Bethesda MD.

Over 150 GWA studies have implicated SNPs influencing a wide array of common diseases and traits, but characteristics of these SNPs have not been systematically examined. For GWA studies published through May 8, 2008 and attempting to assay at least 100,000 SNPs, we identified up to five replicated but previously unreported SNP-trait associations at $p < 9.5 \times 10\text{-}6$. Estimates of risk allele frequencies, sample sizes, and odds ratios (OR) were extracted from the published reports; attributable risk percents (AR%) were calculated from published ORs. Genomic characteristics of these SNPs were retrieved from dbSNP, the HapMap website, and the UCSC Genome Browser. Genetic distances were estimated for each SNP for the three main HapMap populations. Of 284 unique SNPs identified, 139 (49%) were in genic regions. Fourteen SNPs (5%) were missense variants, 3 (1%) were synonymous coding variants, 2 were in a 5 UTR, 2 in a 3 UTR, and 118 (42%) were intronic. Median genetic distances between the HapMap YRI and CEU populations, and between YRI and JPT+CHB, were 0.023 and 0.028, respectively; 95th percentile values were 0.16 and 0.192, respectively. Reported risk allele frequencies ranged from 5%-97% (median 41%); only 3.5% of risk alleles were present at frequencies of 10% or lower. For SNPs associated with binary outcomes, reported ORs ranged from 0.43 to 20.1 (median 1.27), which translated into AR% of 3%-95% (median 22%). Several complex traits of public health interest were represented among those with AR% > 50%, including diabetes and stroke. Though SNPs assayed on genotyping platforms are likely to be overrepresented here, these results suggest that a systematic review of SNPs implicated in GWA studies may yield useful insights into the nature of genetic variation influencing complex traits. Additionally, the modest to strong increase in disease risk attributable to several genetic variants may guide future studies that assess whether these variants, or variants in linkage disequilibrium, are of clinical or public health significance.

Primary torsion dystonia due to Tor1A GAG deletion in 3 Turkish Patients. *U. Yilmaz[1], FB. Atac[2], D. Yuksel[1], H. Verdi[2], D. Yilmaz[1], N. Senbil[1]* 1) Dr.Sami Ulus Pediatrics Hospital, Ankara, Turkey; 2) Dept Medical Bio and Genetics, Baskent Univ Fac Medicine Ankara, Turkey.

Primary torsion dystonia (PTD) is a clinically and genetically heterogeneous movement disorder which is characterized by involuntary sustained muscle contractions. The 3-bp (GAG) deletion in the TOR1A (DYT1) gene was found in patients with PTD linked to 9q34. In this study, 3 patients who have attended to our clinic with dystonic symptoms and have demonstrated GAG deletion in DYT1 gene have been described. In these 3 patients although the diagnosis have been done when they were in 9, 9 and 12 year-old, their symptoms started respectively at the age of 3, 7 and 2. The symptoms began focally at the right hand in two patients and multifocally in both hands in the third patient. None of the patients had a family history and their parents were not relatives. In their initial examination, except dystonia there was not any other pathologic finding. All the patients cranial magnetic resonance imagings, serum copper - ceruloplasmin levels, urine - blood aminoascites and urine organic ascite levels were normal.The results of the molecular analyisis reveals the existance of GAG deletion in TOR1A gene. Their fundoscopic examination were also normal. During two year follow-up period, the patient with multifocal beginning had no progression in his symptoms and had benefit from anticholinergic therapy. In one of the patients who had a focal beginning at the right hand, the dystonia spread to the left arm and in the other patient spread to the right leg and inspite of various therapies these patients didnt show any significant improvement. This finding is in harmony with the articles reporting that the primary dystonia with juvenile onset has a more rapid progression than the adult onset primary dystonias. Although there have not been large case series in DYT1 primary torsion dystonia, the average beginning age have been reported as 11. In our patients it has been observed that the beginning age of the dystonic symptoms were younger.

Development of specific biologic tests for B and T immune response against type VII collagen: tools for gene therapy in RDEB patients. *V. Pendaries[1], G. Alberola[1], M. Titeux[1], C. Leroux[2], Z. G. Vitezica[1], J. Mejia[1], A. Décha[1], C. Prost[2], A. Hovnanian[1,3]* 1) U563, Inserm, Toulouse, France; 2) Avicenne Hospital, Bobigny, France; 3) Purpan Hospital, Toulouse, France.

Dystrophic epidermolysis bullosa (DEB) is a severe inherited skin disorder caused by loss-of-function mutations in COL7A1 encoding type VII collagen, the component of anchoring fibrils. In the auto-immune blistering disease, epidermolysis bullosa acquisita (EBA), circulating autoantibodies are produced against type VII collagen, while patients with vesiculobullous systemic lupus erythematosus (VBSLE) also display antibodies against this protein. To assess the immunologic risk in recessive DEB patients during gene therapy restoring type VII collagen expression, we developed ELISA and ELISPOT assays using the full-length type VII collagen molecule. Biochemical analysis of secreted type VII collagen demonstrated that this protein assembled into trimers and adopted a triple-helical conformation. The ELISA and ELISPOT assays were performed on a cohort of 44 untreated EBA and VBSLE patients. The ELISA assay detected type VII collagen circulating antibodies with high specificity (95%), sensitivity (65%) and predictive positive value (91%). The ELISPOT assay detected a Th1 immune response against type VII collagen, which correlated with disease activity. We subsequently tested 4 RDEB patients. The ELISA assay was positive for anti-type VII collagen antibodies in one of the 2 patients who did not express detectable type VII collagen. The two patients who expressed low levels or a truncated form of type VII collagen were non-responsive in the ELISPOT assay. Conversely, a positive Th1 immune response was observed in the 2 RDEB patients who expressed no type VII collagen. HLA typing of EBA and RDEB patients is in progress to determine if a specific HLA subtype is a risk for developing an immune response against type VII collagen. The immunologic tests for the B and T immune response to type VII collagen that we have developed will be useful to select candidates for clinical trials of gene therapy for RDEB. They will also be essential to the immunological follow-up of treated RDEB patients during these trials.

Fetal Constraint as a Potential Risk Factor for Craniosynostosis. *P. A. Sanchez-Lara[1], S. L. Carmichael[2], J. M. Graham, Jr.[3], E. J. Lammer[4], S. A. Rasmussen[5]* 1) Childrens Hospital Los Angeles, Los Angeles, CA; 2) California Research Division, March of Dimes, Oakland, CA; 3) Medical Genetics Institute, Cedars-Sinai Medical Center; 4) Childrens Hospital Oakland Research Institute, Oakland, CA; 5) Centers for Disease Control and Prevention, Atlanta, GA.

Fetal head constraint has been hypothesized to contribute to the risk of craniosynostosis(CS). We used data from the National Birth Defects Prevention Study to evaluate for an association between CS and factors potentially indicative of fetal constraint which included multiple gestation (twins or higher), macrosomia (birth weight >4000 g), post-term gestational age (>42 weeks), and parity (nulli- or primiparity). Case infants (n=677) had CS documented either by radiographic evidence or by surgical intervention, excluding those with known or suspected genetic conditions. Control infants (n=5,958) had no major birth defects and were randomly selected from the same population as case infants. Logistic regression estimated the odds ratios for association, while adjusting for several covariates (maternal age, race-ethnicity, education, body mass index, fertility treatments and parity, paternal age, and infant sex). The reported adjusted odds ratios (aOR) includes adjustment for the above covariates and exclusion of certain subjects. Specifically, for plurality, we excluded subjects whose mothers had fertility treatments or pre-pregnancy or gestational diabetes, and for the remaining exposure variables, we excluded subjects from multiple gestations and subjects whose mothers had pre-pregnancy or gestational diabetes. Results: multiple gestation (compared to singletons), aOR 1.7, 95% CI 0.9-3.0;, macrosomia (compared to normal birth weight), aOR 1.1 95%, CI 0.9-1.5; prolonged compared to term gestation, aOR 1.1, 95% CI 0.6-2.3; and no and one previous live birth, compared to two or more, aOR 0.9, 95% CI 0.7-1.2, and aOR 0.9, 95% CI 0.7-1.2, respectively. A lack of dural stretch has been proposed as a mechanical signal for premature suture closure. We found that the studied factors- multiple gestation, macrosomia, prolonged gestation and nulliparity-were not associated with CS, after adjusting for potential confounders.

Genetic variation in folate pathway enzymes exhibit sex-specific association in cases of Down syndrome-associated congenital heart defects. *A. E. Locke[1], E. G. Allen[1], S. W. Tinker[1], K. J. Dooley[2], G. Capone[3], C. Cua[4], E. Feingold[5], S. L. Sherman[1], L. J. H. Bean[1]* 1) Dept of Human Genetics, Emory University, Atlanta, GA; 2) Sibley Heart Center Cardiology, Children's Hospital of Atlanta, Atlanta, GA; 3) Division of Neurology and Developmental Medicine, Kennedy Krieger Institute, Baltimore, MD; 4) Nationwide Children's Hospital, Columbus, OH; 5) Depts of Human Genetics and Biostatistics, University of Pittsburgh, Graduate School of Public Health, Pittsburgh, PA.

Folate is a nutrient vital for nucleotide synthesis, DNA and protein methylation, and homocysteine to methionine conversion. Maternal folate deficiency has been associated primarily with neural tube defects, but also with other birth defects including heart defects. Additionally, numerous studies have associated maternal polymorphisms in folate pathway genes with the risk of having a child with Down syndrome (DS). We hypothesized that folate pathway polymorphisms contribute to the high incidence of heart defects observed in fetuses with Down syndrome. These effects could be exaggerated in females with DS who have a higher methylation burden and, as we have shown, an increased risk of AVSD. Cases defined as individuals with DS and complete atrioventicular septal defect (AVSD) (93 white, 29 black) were compared to controls, defined as individuals with DS with no heart defect (94 white, 28 black). SNPs in the folate pathway genes MTHFR, MTR, MTRR, and CBS as well as the reduced folate carrier gene SLC19A1 were gentoyped. Consistent with our hypothesis transmission of the c.2756AG MTR G allele (rs1805087) was significantly associated with the risk of AVSD in families with a female, but not a male proband ($p = .002$). Transmission of SNPs within the MTHFR gene show evidence for complex survival factors that will be further investigated. Statistical methods to make genotype calls from Illumina data on trisomic chromosome 21 SNPs in CBS and SLC19A1 have been developed and the potential role of polymorphisms in these genes contributing to the risk of AVSD is being evaluated. Epidemiologic data will be used to explore the relationship between dietary folate, genotype, and AVSD susceptibility.

Potential interactors of PHOX2B as candidate genes for orphan central hypoventilation and companion disorders. *S. Parodi[1,2], T. Bachetti[1], S. M. Kooistra[3], R. Ravazzolo[1,2], I. Ceccherini[1], B. J. L. Eggen[3]* 1) Lab di Genetica Molecolare, Istituto Giannina Gaslini, Genova, Italy; 2) Dipartimento di Pediatria, Università degli Studi di Genova, Genova, Italy; 3) Developmental Genetics, Groningen Biomolecular Sciences and Biotechnology Institute,University of Groningen, Haren, Netherlands.

The PHOX2B gene encodes a 314 amino acids paired box homeodomain transcription factor known to play a key role in the development of the central and peripheral autonomic nervous system. Accordingly, Phox2b-/- mice die in utero with absent autonomic nervous system circuits, as those neurons either fail to form or degenerate. In particular, Phox2b has been implicated in the specification of neuronal subtypes, acting as determinant of the noradrenergic phenotype. Mutations of the PHOX2B gene have recently been associated with Congenital Central Hypoventilation Syndrome (CCHS or Ondines curse), a life-threatening disorder characterized by impaired autonomous breathing and other autonomic dysfunctions such as neuroblastoma and Hirschsprung disease. Although the function of this trascription factor in the noradrenergic neuron differentiation is well studied, the PHOX2B pathway remains poorly understood. In order to identify interaction partners of PHOX2B, we carried out a yeast two-hybrid screen using a 11 day mouse embryo pretransformed cDNA Library. Among many interacting clones obtained, we focused on proteins known to localize in the nucleus, to modulate transcriptional activities or to act as chromatin remodelling factors. In particular, we are evaluating, by co-immunoprecipitation and co-localization assays, an actin related protein, specifically expressed in the brain and in differentiated neurons and a nuclear factor whose knockout mouse model dies at postnatal day 1 exhibiting failure to suckle, cyanosis and respiratory distress. Indentification of PHOX2B interactors represents an useful step towards the reconstruction of a full genotype-phenotype correlation in CCHS as well as an alternative way to discover new candidate genes for orphan central hypoventilation and companion disorders.

GENETIC ANALYSIS AND EPIDEMIOLOGICAL DISTRIBUTION OF SPANISH SPINOCEREBELLAR ATAXIAS. *V. Volpini[1], J. Corral[1], I. Banchs[1], L. De Jorge[1], H. San Nicolas[1], O. Combarros[2], J. Berciano[2], C. Serrano[3], M. Calopa[4], A. Matilla[5], D. Genís[6]* 1) Center for Molecular Genetic Diagnosis of Hereditary Diseases, CDGM-IDIBELL, L'Hospitalet de Llobregat, Spain; 2) Dept. of Neurology, Hosp.Univ. Marqués de Valdecilla, Santander, Spain; 3) Dept. of Neurology, Hosp. Sant Joan de Déu, Martorell, Spain; 4) Dept. of Neurology, Hosp. Univ. de Bellvitge, L'Hospitalet de Llobregat, Spain; 5) Health Sciences Research Institute Germans Trias i Pujol, Badalona, Spain; 6) Dept. of Neurology, Hosp. Univ. Josep Trueta, Girona, Spain.

Autosomal dominant cerebellar ataxias (ADCA) are a clinically and genetically heterogeneous group of neurodegenerative disorders in which several spinocerebellar ataxia (SCA) genes have been cloned: SCAs1-3, SCAs6-7, SCA12 and SCA17; sharing a CAG repeat expansion mutations which generally encodes a polyglutamine tract. In SCA8 the mutation is an untranslated CTG repeat. We have analyzed 353 unrelated familial and 1,043 sporadic and idiopathic cases of SCA. Over the familial cases 6.00% were SCA1; 26.67% SCA2; 33.33% SCA3; 7.33% SCA6; 6.67% SCA7; 14.67% SCA8 and 1.33% SCA17. In 22 familial index cases with SCA8 expansions the allele range goes from 85 to 470 repeats (129.32%67.55%; Pearson Coef.=52.24%). Maternal transmissions presented elongations of the CTG combined sequence ranging from +2 to +11 repeats (5.334.93; Pearson Coef.=92.49%). In contrast, paternal transmissions presented contractions ranging from -1 to -17 repeats (-9.756.89; Pearson Coef.=-70.75%). Several giant SCA8 expansions ranges from 401 to 1126 (N= 9), carried by unaffected adult individuals and being originated from homozygous SCA8 females with alleles of moderate size. In contrast,the homozygous males have transmitted contracted alleles, as in heterozygous cases occurs. We have tested 90 individuals from general population and the distribution of SCA8 alleles could be classified in two groups: 15 to 34 CTGs with frequency 98% and 77 to 86 CTGs with frequency 2%. About 60% of familial ADCA cases remained genetically unclassified. No SCA mutations were detected in the 1,043 isolated and idiopathic cases of spinocerebellar ataxia.

GENETIC INTERACTIONS BETWEEN 2 OLIGOGENIC DISEASES: BARDET-BIELD SYNDROME AND HIRSCHSPRUNG DISEASE. *L. de Pontual[1], S. Thomas[1], H. Dolffus[2], C. Baumann[3], S. Audollent[1], A. Pelet[1], N. Katsanis[4], P. Beales[5], A. Munnich[1], H. Etchevers[1], S. Lyonnet[1], T. Attie-Bitach[1], J. Amiel[1]* 1) Dept Genetics, Hosp Necker-enfants Malades, Paris, France; 2) Service de Génétique, Hôpital de Haute-Pierre, Strasbourg, France; 3) Service de Génétique, Hôpital Robert Debré, Paris, France; 4) McKusick-Nathans Institute of Genetic Medicine, Baltimore, Maryland; 5) Molecular Medicine Unit, London, United Kingdom.

Bardet-Biedl syndrome (BBS, MIM 209900) is an autosomal recessive multisystemic disorder genetically heterogeneous with 13 BBS genes hitherto identified. BBS proteins are involved in primary ciliary and basal body function, affecting both transport and signalling of the planar cell polarity pathways. BBS patients are predispose to Hirschsprung disease (HSCR, MIM 164761) as it is reported in 5 to 10% of the cases versus 1/5000 in the general population.RET is the major gene in HSCR. We genotyped the hypomorphic allele located in a conserved non-coding sequence in intron 1 of the RET gene in 54 BBS patients with or without HSCR. In this series, mutations in BBS 1- 2- 4- 5- 7 and 10 have been identified, and no correlation between the genotype and the phenotype could be drawn as espected by the observation of intrafamilial variability of expression. We report an over-transmission of the RET hypomorphic allele in sporadic BBS+HSCR cases and we show that the RET hypomorphic T allele can account for the variability of HSCR expression within BBS families. We then demonstrated co-segregation of both BBS gene mutations and the RET hypomorphic allele HSCR patients only within BBS familial cases. Moreover, we identified a 11bp deletion encompassing a DNA binding site for the transcription factor SNAIL (will be confirmed by ChiP) in a RET enhancer in a sporadic case of BBS+HSCR. These data strengthen the role of the RET gene in the co-occurrence of BBS and HSCR and, suggest genetic interactions between two oligogenic disorders. Functional studies have been undertaken to demonstrate a possible role of BBS proteins on human neural crest cells migration.

Pure partial trisomy 19p : identified by MLPA subtelomeric screening. *H. Yoshihashi, N. Furuya, K. Kurosawa*
Dept Med Gen, Kanagawa Children's Med Ctr, Yokohama, Japan.

Submicroscopic subtelomeric chromosomal rearrangements are considered to be a significant cause of idiopathic mental retardation (MR). Unbalanced rearrangements involving chromosome 19, which has a greater density of genes than any other human chromosome, are expected to have serious clinical implications. Reports of rearrangements involving chromosome 19 are quite uncommon. In particular, few malformed individuals with partial trisomy 19p accompanying partial monosomy of different chromosomes have been reported. Both participating choromosomes contribute to the phenotype, producing complex malformation patterns. Here, we report on an infant with pure partial trisomy 19p. Case: The propositus was a 3-year-old girl. At 30 weeks of gestation, she was born by Cesarean delivery due to fetal asphyxia. Birth weight was 826 g (-2.7SD), length 32 cm (-2.9SD), and head circumference 23 cm (-2.7SD). She had growth retardation(IUGR), developmental delay, and distinctive facial appearance (telecanthus, sparse hair, micrognathia, long philtrum, bulbous nose). Standard karyotype was normal. Cytogenetic study: Multiplex-Ligation Probe Amplification (MLPA) has come into wide use for subtelomeric screening as a new molecular cytogenetic technique. Subtelomeric assay using MLPA with SALSA P036B and P070 kits (MRC-Holland) revealed only gain of the short arm of chromosome 19. The metaphase FISH with a telomere probe specific for 19p and 19q (Vysis, Downers, Grove, IL) showed that the extra 19ptel signal was present at the short arm of chromosome 13. Based on our FISH mapping analysis, we estimate the size of trisomic region from 19p13.3 to be approximately 3.0-3.8Mb. The parents had normal karyotype and showed no abnormal hybridization results. The duplication of 19ptel was confirmed as de novo subetelomeric imbalance. Summary: We review four reports of individuals with partial trisomy for terminal 19p rearrangements (Byrne et al.,1980; Salbert et al.,1992; Brown et al., 2000; Quigley et al., 2004). To our knowledge, the present case is the first report of pure partial trisomy 19p and contributes to a bettter clinical characterization of duplication of 19ptel.

Lethal hypophosphatasia with spina bifida, a diagnostic delimma. *H. I. Alzaidan[1], M. Alowain[1], D. Arafah[1], H. Abalkhail[2]* 1) Medical Genetics, King Faisal Specialist Hospital and Research ctr, Riyadh, Riyadh-11211, Saudi Arabia; 2) Pathology department, King Faisal Specialist Hospital and Research ctr, Riyadh, Riyadh-11211, Saudi Arabia.

Hypophosphatasia is an autosomal recessive disorder characterized by a defect in mineralization of bone with low activity of serum and bone alkaline phosphatase (ALP). There are different forms of clinical presentation according to the age of onset and severity of the disease. We report a newborn of a consanguineous couple who died shortly after birth because of severe pulmonary insufficiency. He was labeled initially by prenatal U/S to have (lethal skeletal dysplasia). examination revealed dysmorphic newborn with large head, absence of the skull bones, short thick extremities and lumbosacral myelomeningocoele. The diagnostic thinking process represents a great prototype of the diagnostic challenge that dysmorphologist and skeletal dysplasia experts face while approaching such cases. Chromosomal analysis showed normal male karyotyping; babygram revealed very abnormal limbs with short, poorly formed and demineralized bones, large head with deficient ossification of the skull vault. Appearance was suggestive of lethal osteogenesis imperficta(OI) as per our radiologist. Skin biopsy was sent initially for COL1A1 and COL1A2 the only known genes for autosomal dominant OI and came back negative for mutations. Then we considered cartilage associated protein CRTAP as a potential cause at that stage which is rarer, recently described cause for AR lethal OI. however discussing the X rays findings with the experts in the field of dysplasia raise the possibility of AR Hypophosphatasia as cause. this turned to be the case when we found a homozygous previously reported c.977GT transversion in exon 9 of alkaline phosphatase ALPL gene. It take us more than a year to diagnose the case. We report this case to document the association between Myelomeningocoele and hypophosphatasia which is "up to my knowledge" not reported before. Furthermore, we want to stress the need to include alkaline phosphatase in any case of lethal dysplasia. This probably will save the physician a lot of precious time and effort.

Difficulty of clinical diagnosis of Japanese children with Marfan syndrome. *H. Kawame[1], S. Yasukochi[2]* 1) Div Medical Genetics,; 2) Dep Cardiology, Nagano Children's Hospital, Nagano, Japan.

Marfan syndrome (MFS) is a systemic connective tissue disorders caused by mutations in the FBN1 gene. It is characterized with variety of skeletal, cardiovascular, ocular manifestations. Aortic root aneurysm and dissection are major cause of mortality. Early diagnosis is crucial for appropriate management including medical therapy of beta-adrenergic blocker and potential drug, Losartan, and prophylactic surgical procedures. The diagnosis of MFS is based clinical ground according to the Ghent criteria. But application to childhood MFS is challenging, because of age-dependent nature of manifestations. Also standards for clinical features such as dolichostenomelia have not been applicable for Asian ethnic population. In order to evaluate the phonotypic spectrum and value of Ghent criteria for Japanese children with MFS, we reviewed 13 children (age range: 2 - 16 years) with MFS or suspected MFS referred to our Genetic clinics in Nagano Childrens Hospital from 2002 to 2007. Seven were familial cases.

Four (5, 7, 11, 11 years) familial cases and one sporadic case (16 years) (38%) fulfilled the Ghent criteria. In skeletal system, all had involvement and no patient presented major criteria. None had arm span-to-height ratio more than 1.05. All had joint hypermobility and no reduced extension at the elbows. In cardiovascular system, 4 (30%: 5, 11, 11, 13 years) had major criteria and 2 (15%) had involvement. One patient had ectopia lentis (7 years). No patient had pelvic X-ray for protrusio acetabuli, and MRI or CT for dual ectasia. Although three patients had only involvement in the skeletal system, all were diagnosed as emerging MFS and offered repeat evaluations. In summary, Ghent criteria has serious limitations in the diagnosis of Japanese children with MFS. It seems appropriate to consider children with Marfan-like features as emerging MFS, and offer them periodical evaluations with psychosocial supports to the parents. These findings indicated genetic testing including FBN1, TGFR1 and TGFR2 genes is recommended in children with suspected MFS.

Molecular modeling of NIPBL missense mutations: an adjunct tool for the comprehension of genotype-phenotype correlations. *S. Ferraiuolo[1], M. Masciadri[1], C. Gervasini[2], P. Castronovo[2], A. Selicorni[3], D. Milani[3], L. Larizza[2], S. Russo[1]* 1) Molecular Laboratory, Istituto Auxologico Italiano, Milano, MI, Italy; 2) University di Milano - Osp San Paolo- MI-Italy; 3) Clinica De Marchi- University of Milano - Italy.

Cornelia de Lange syndrome (CdLS) is a rare multisystem disorder characterized by facial dysmorphisms, upper limb abnormalities, growth and cognitive retardation. The main causative gene, NIBPL, is responsible for CdLS in about 50% of patients; the encoded protein,delangin,belongs to the adherin family and is involved in sister chromatid cohesion, DNA repair and long range gene regulation. Within a cohort of 97 CdLS patients with a phenotype scored as severe, moderate or mild, we identified 46 NIPBL mutations, including 8 missense mutations. Genotype-phenotype correlation is not clearly defined for NIPBL mutations, in particular the missense ones,which appear to be associated with a variable phenotype. Aiming at establishing if a molecular modeling approach might be in keeping with the clinical presentation of carriers of missense mutations, we addressed the modelling of NIPBL protein. Indeed the X-ray structure has been deposited in PDB only for the second part of the protein. We built up a 3D model of the human NIPBL protein using the Fold Recognition approach. Several different structural and evolutionary criteria enabled us to choose as model the QBK1 Rod-like C-shape structure as it showed the best score following validation (VERIFY3D) and energetic minimization (GROMACS). Up to day in silico mutagenesis of three substitutions was carried on by this model: p.Arg1856Gly associated with a very severe clinical score, p.Arg2298Leu and p.Arg2298Cys both affecting the same residue and underlying a moderate clinical phenotype. Only Arg1856Gly affects the folding region, losing crucial intramolecular interactions and exposure to the solvent, while the other two variants cause mild conformation changes.These preliminary results indicate the usefulness of bioinformatic tools in the comprehension of genotype-phenotype correlation. Evaluation of other missense mutations mapping within the modeled region is in progress.

Estimating The Coefficient Of Linkage Disequilibrium Between A QTL And A Marker Locus. *S. Ghosh* Human Genetics Unit, Indian Statistical Inst, Kolkata, India.

Although statistical methods based on case-control designs are the most popular and extensively used approach for genetic association mapping of binary traits, development of such methods for quantitative traits is currently an active area of interest. While there are some classical approaches like analysis of variance as well as novel approaches based on quantiles of the quantitative trait for testing for association, these methods do not involve the estimation of the coefficient of linkage disequilibrium between the putative QTL and the marker locus. We propose an estimation procedure of this parameter based on the Expectation-Maximization (EM) algorithm and simultaneously develop a test for association. We assume that the quantitative trait is controlled by a biallelic QTL and is distributed as normal conditioned on the genotypes of the QTL. The estimation involves a two-stage procedure. In the first stage, we estimate the posterior probabilities of the QTL genotypes conditioned on the quantitative trait values from a mixture of three normal populations. In the second stage, we estimate the two-locus haplotype frequencies based on the QTL and the marker genotypes using the posterior probabilities of the QTL genotypes obtained in the first stage. We test for association using the resultant estimate of the coefficient of linkage disequilibrium using permutation principles. We note that the parameters of the component normal distributions in the mixture are nuisance parameters and are not used in either the second stage of our estimation procedure or for testing for association. We perform Monte-Carlo simulations for different genetic parameters and probability distributions of the quantitative trait to assess the efficiency of the estimator and the power to detect association. We find that method is sufficiently robust to lack of normality of the quantitative trait values, although one can improve the efficiency by classical transformations like logarithmic or square root.

Miglustat in patients with Niemann-Pick Type C disease (NPC): a multicentre retrospective survey. *J. E. Wraith[1], M. Pineda[2], F. Sedel[3], W. L. Hwu[4], M. Rohrbach[5], B. Bembi[6], G. C. Korenke[7], R. Giorgino[8], P. Schieber[8], M. C. Patterson[9]* 1) Royal Manchester Children's Hospital, UK; 2) Hospital Sant Joan de Deu, Barcelona, Spain; 3) Hopital Pitié Salpêtrière, Paris, France; 4) National Taiwan University Hospital, Taipei, Taiwan; 5) Kinderspital, Zürich, Switzerland; 6) Regional Coordinator Centre for Rare Diseases, University Hospital, Udine, Italy; 7) Elisabeth Kinderkrankenhaus, Oldenburg, Germany; 8) Actelion Pharmaceuticals Ltd, Allschwil, Switzerland; 9) Mayo Clinic, Rochester, USA.

A clinical trial indicated that miglustat is able to slow disease progression in patients with NPC.[1] We present the interim results from an ongoing international, retrospective survey assessing neurological disease progression in NPC patients treated with miglustat. All NPC patients prescribed miglustat in 17 selected expert centres are included. Treating physicians complete a questionnaire on patient demographics, treatment history, disease progression and general health. A disease-specific disability scale[2] evaluates dysphagia, dystonia, ataxia and dysarthria severity at diagnosis, treatment initiation and last visit. As of 17 December 2007, 44 patients were included. Median (range) miglustat exposure was 17.4 (0.6-40.2) months. Of 43 evaluable patients (meanSD age: 9.97.2 years at diagnosis, 14.29.5 years at miglustat initiation), most remained at least stable after treatment as regards dysphagia (n=36; 84%), dystonia (n=33; 77%), ataxia (n=31; 72%) and dysarthria (n=31; 72%). Thirty-two (74%) patients were classified as good responders, with at least 3 out of the 4 scale parameters rated as stable or improved. According to physician global assessments, 16/41 (39%) patients improved in general health, and 30/38 (79%) patients experienced good/fair benefit. Physicians' intention to maintain patients on therapy was recorded for 31/33 (94%) patients. Miglustat appears to provide clinically relevant benefits on neurological disease progression in patients with NPC. These data are consistent with results from a previous clinical trial[1] in the same patient population. [1]Patterson et al. Lancet Neurol 2007;6:765-72. [2]Iturriaga et al. J Neurol Sci 2006; 249:1-6.

Active chromatin marks on ultraconserved non-coding elements. *U. Choudhury[1,3], F. Parisi[2], F. Naef[2], SE. Antonarakis[1]* 1) Gen Med, Univ Geneva Medical School, Geneva, Switzerland; 2) School of Life Sciences, Polytechnic Federal School of Lausanne, Lausanne, Switzerland; 3) NCCR Frontiers-in-Genetics Doctoral School, University of Geneva, Geneva, Switzerland.

Identifying gene regulatory elements and unraveling their mechanisms of action is a major challenge in the field of genomics. Sequence conservation between species is an efficient criterion for finding such elements. Ultraconserved elements (UCEs) are sequences with extreme conservation levels between mammals. They are 100% identical between human, mouse and rat over at least 200 bp. About 2/3 of UCEs are non-coding and map in the vicinity of genes that are key regulators of vertebrate development. Regulatory elements are known to possess specific chromatin signatures. We analyzed the chromatin state of 286 non-coding UCEs by ChIP-on-chip using antibodies against histone H3 modifications that mark either active chromatin (K4-dimethyl/trimethyl) or repressed chromatin (K27-trimethyl and K9-dimethyl) in different human cell types (lymphoblastoids, fetal lung and neuroblasts). Immunoprecipitates were hybridized to a custom tiling array containing a 2kb region around each UCE, promoter regions of the genes nearby and control sequences located upstream and downstream of each UCE. A statistically significant fraction of the UCE regions showed enrichments for H3 K4-dimethyl and K4-trimethyl modifications (30 and 10%, respectively). Interestingly, the modified histones were generally positioned close to rather than over the UCE sequence. In most cases, enrichment for K4-trimethyl was not linked to the presence of a known transcriptional start site (26 versus 9 regions), although this mark is reported to be tightly associated to the 5 end of actively transcribed genes. The presence of active chromatin marks on UCE regions strengthens the hypothesis of a gene regulatory activity of these elements and sheds light on their profile of activity. Our results provide a framework for further investigating their mechanisms of action and testing for the presence of non-coding RNAs in the relevant cell lines.

Mutations in *Frem1* yield an animal model of retrosternal diaphragmatic hernia. *D. A. Scott[1], M. Wat[1], B.-J. Kim[1], O. Shchelochkov[1], D. W. Stockton[2], M. J. Justice[1], B. Lee[1,3]* 1) Dept Molecular & Human Genetics, Baylor College of Medicine, Houston, TX; 2) Dept Pediatrics, Wayne State University School of Medicine, Detroit, MI; 3) Howard Hughes Medical Institute.

Congenital diaphragmatic hernia (CDH) is a common sporadic birth defect that affects ~1:3000 newborns. Anterior diaphragmatic hernias account for approximately 10% of CDH cases. These hernias are often referred to as Morgagni hernias since the majority involve herniation through the foramina of Morgagni, two small parasternal regions between the costal and sternal attachments of the diaphragm. However, some hernias affect the region directly behind the sternum and are best described as retrosternal in nature. In a recessive ENU mutagenesis screen we identified a mouse strain with several congenital anomalies including retrosternal diaphragmatic hernia, lung segmentation defects and unilateral kidney agenesis. All cases of CDH involved herniation of a portion of the liver and, in some cases, a hernial sac consisting of a thin adherent membrane was also present. Traditional linkage mapping followed by sequencing of positional candidate genes revealed a homozygous 1687A>T (I563F) change in the extracellular matrix protein *Frem1*. This *Frem1* mutation is likely to be causal since these mice showed failure of complementation when crossed to mice carrying an L826X mutation in the same gene. Further studies revealed that the penetrance of both the retrosternal CDH and the lung segmentation defects were highly dependant on genetic background. We note that incomplete penetrance has also been a hallmark of human CDH suggesting the importance of genetic modifiers. Although human CDH can affect any portion of the diaphragm, data from this and other recently identified CDH mouse models*Gata4*, anterior CDH; *Slit3*, central CDH; *Coup-TFII*, posterolateral CDHsuggest that abnormal formation of one region of the diaphragm may be associated with defects in genes and/or genetic pathways that are distinct from those affecting other regions. Future studies will be aimed at understanding the mechanisms by which *Frem1* affects retrosternal diaphragm development and identifying genes that modify the *Frem1* phenotype.

Use of Public Genotype Data from Multiple Platforms to Increase Power of a Genome-wide Association Study of Parkinson's Disease. *A. J. Oudes, E.Sasha Paegle* Application Science, Rosetta Biosoftware, Seattle, WA.

Recently, genome-wide association (GWA) studies have been conducted to investigate several diseases including type 1 and 2 diabetes, prostate cancer, breast cancer, and heart disease. These studies identified numerous loci associated with predisposition for these diseases. Other GWA studies investigating asthma and Parkinson's disease have found few if any significantly associated loci. An important consideration when designing a GWA is study power. One way to increase study power is to add genotype data from unaffected individuals. Repositories such as dbGAP and the Illumina iControl database are sources of genotype data that may be used to increase power of GWA studies. However, the use of these data in a secondary analysis is challenging due to issues such as population stratification, cryptic relatedness, and lack of corresponding markers between genotyping platforms. We used Parkinson's disease GWA data from Fung et al. in combination with Illumina iControl data studies 64 and 67 to increase the power of the original study. To assess the structure of the composite control population, we used multi-dimensional scaling to identify and subsequently remove outlier individuals. Analysis of the powered study revealed previously unidentified loci on chromosome 2 that were associated with the disease state. To address the lack of corresponding markers between data sets, a linkage-disequilibrium based "clumping" analysis of association results was performed between our powered results and those from the LEAPS Parkinson's GWA (Maraganore et. al.). Clumping analysis identified differing disease associated SNPs within 250 Kb windows, which corresponded between the studies at a p-value of <0.01. In conclusion, we used public genotype data to increase the power of an existing GWA study and identified novel Parkinson's disease associated alleles. Comparison of the powered results with those from a previously published study identified corresponding disease associated alleles.

G-protein-coupled receptor kinase 5 and dementia in Parkinsons disease. *P. Tarantino[1,2], E. V. De Marco[1], F. E. Rocca[1,3], F. Annesi[1], D. Civitelli[1], G. Provenzano[1,2], V. Scornaienchi[1], V. Greco[1], G. Annesi[1]* 1) Inst Neurological Sci, National Research Council, Mangone, Cosenza, Italy; 2) Departement of Neurosciences, Psychiatry and Anaesthesiology University of Messina, Messina-Italy; 3) Institute of Neurology, University Magna Graecia, Catanzaro-Italy.

Parkinsons Disease (PD) is characterized by selective degeneration of dopaminergic neurons in the substantia nigra. Dementia is a common complication of PD. Although the pathogenesis of the disease is unclear, phosphorylation of alpha-synuclein and its oligomer formation seem to play a key role. G-protein coupled receptor kinase (GRKs) constitute a serine/threonine kinase family. It has recently been demonstrated that GRK5 promotes the formation of soluble oligomers and aggregates of -synuclein. We have performed a case control study to investigate whether genetic variability in the GRK5 gene may predispose to dementia in PD. A total of 114 demented patients and 114 non demented patients with sporadic PD were included in the study. Two polymorphic loci in the GRK5 gene were examined. Cognitive conditions were assessed by Mini Mental State Examination. SNP genotyping was performed by using allelic discrimination on ABI PRISM 7900-HT. All SNPs showed high genotyping quality and were in Hardy-Weinberg equilibrium in both demented and non-demented groups. The haplotype analysis was performed using the software Unphased. We observed association with the two polymorphisms. There were significant differences in the genotype or allele frequencies between the demented and non-demented patients. Furthermore we found significant differences in the inferred haplotype frequencies of the two groups ($p<0.001$). The substrate of dementia in PD is Lewy bodies, mainly costituited of alpha-synuclein. Quite recently, GRK5 have been demonstrated to phosphorilate alpha-synuclein at Ser 129 and other synuclein isoforms. Here, we report genetic evidence of significant association of GRK5 polymorphisms with dementia in PD and therefore it is possible to speculate that the gene has a crucial role in the development of this PD complication. Further studies of other regions of the GRK5 gene are in progress to confirm this association.

Cardiac Transplantation in a Child with Recurrent Thrombosis due to Congenital Thrombophilic Mutations. *S. Ozkan[1], FB. Atac[2], H. Verdi[2], S. Ozcobanoglu[1], E. Uguz[1], A. Sezgin[1], N. Ozbek[3]* 1) Dept Cardiovascular Surgery, Baskent Univ, Ankara, Turkey; 2) Dept Medical Biol and Genetics, Baskent Univ, Fac Medicine, Ankara, Turkey; 3) Dept Pediatric Hematology, Baskent Univ, Fac Medicine, Ankara, Turkey.

Thrombosis is known to be one of the most serious complications leading to increased morbidity and graft dysfunction after solid organ transplantation. Therefore predicting the risk of thrombosis before the operation is important for preventing the complications during posttransplantation follow up. Here we report a cardiac transplantation patient who had Blalock-Taussig (BT) shunt three times. He was heterozygous for two thrombophilic mutations; MTHFR C677T and FV A4070G. We believe, congenital risk factors should be bear in mind in patients who had thromboembolic event(s) before cardiac surgery. Careful application of routine anticoagulation during and after surgery is essential.

A new mitochondrial cytochrome b (MTCYB) gene mutation responsible for a polyvisceral neonatal failure. *K. Fragaki[1,2], V. Procaccio[3], S. Bannwarth[1,2], V. Serre[4], G. Augé[1], A. Figueroa[1,2], F. Casagrande[5], J.-C. Lambert[1], V. Paquis-Flucklinger[1,2]* 1) Service de Génétique Médicale CHU de Nice, France; 2) IGMRC FRE CNRS/UNSA 3086 Faculté de Médecine Nice, France; 3) Department of Pharmacology University of California Irvine, USA; 4) Service de Génétique and INSERM U781 Hôpital Necker Enfants Malades Paris, France; 5) Service de Pédiatrie CHU de Nice, France.

To date, the majority of MTCYB mutations have been reported in patients with severe exercise intolerance. Here we report a child born to non consanguineous parents with no family antecedent. At birth, he presented with hypotonia, respiratory distress and hypertrophic cardiomyopathy. He developed seizures and died 24h later of a polyvisceral failure. Biochemical analysis showed major lactic acidosis and hepatocellular insufficiency. We first proceeded by enzymatic analysis of respiratory chain activity which showed isolated complex III deficiency in liver, whereas all respiratory chain complexes activities were normal in muscle. Western blotting also demonstrated isolated severe loss of complex III expression in liver, whereas all subunits levels in muscle were unaffected. Sequencing of the MTCYB gene, revealed a previously unreported 15635 T>C transition leading to a highly-conserved amino-acid substitution (S297P) located in a transmembrane hydrophobic helix of cytochrome b. RFLP analysis showed that this transition was homoplasmic in all patients tissues studied and undetectable in various cellular types of patients mother. Modelling of this transition showed interruption of the helix due to geometrical constraints induced by proline. Finally, cell fusion experiments between rho0 lymphoblastoid cells, fully depleted of mtDNA, and patients fibroblasts did not led to respiratory chain defect complementation in mitochondria of the resulting cybrid cells, thus demonstrating the mitochondrial origin of the defect initially observed. All these biochemical and molecular elements demonstrate the extreme pathogenic character of this new mutation, which likely occured de novo. These findings also have a major interest for genetic counselling in this family.

Hyperdiploidy with trisomy 9 and deletion of the p16 locus in precursor T-cell acute lymphoblastic leukemia. *J. Morrissette[1], K. Healey[1], A. McKenzie[2], G. Halligan[2], JP. de Chadarévian[1]* 1) Department of Pathology, St Christopher's Hospital for Children, Philadelphia, PA; 2) Department of Pediatrics, Section of Oncology, St Christopher's Hospital for Children, Philadelphia, PA.

We describe the rare finding of a 4 year-old male who presented with T-cell acute lymphoblastic leukemia (T-ALL) and a pre-treatment bone marrow karyotype mosaic for four distinct cell lines. G-band analysis of metaphase cells identified a hyperdiploid cell line (52 chromosomes) in 11/19 cells studied, which were trisomic for chromosomes 6, 9, 11, 13, 19, and 22. Fluorescence in situ hybridization (FISH) analysis demonstrated that these hyperdiploid cells were missing all three copies of the p16 locus at 9p21. FISH analysis of the interphase nuclei also identified cells with homozygous deletions of the p16 locus at 9p21, and containing two chromosome 9 centromere signals in 88/100 cells; another cell line demonstrated a heterozygous deletion with one p16 locus intact in 6/100 cells. A normal signal pattern was found in 6/100 cells. This suggests the presenting bone marrow had undergone clonal evolution, with the most evolved clone seen in the G-banded karyotype hyperdiploid line. Although a non-random pattern of hyperdiploidy, with trisomy for chromosomes 4, 10, 17 and 21, is commonly seen in childhood pre-B ALL cases, the hyperdiploid cell line observed in this patient did not fit this pattern. This case represents a rare case of hyperdiploidy in T-cell ALL, and identifies clonal evolution of the 9p21 deletion.

The importance of gender-specific and non-additive effects in complex traits: insights from a 33000 person genome-wide association study of height. *H. Lango[1], G. Lettre[2], M. N. Weedon[1], GIANT (Genetic Investigation of ANthropometric Traits) Consortium* 1) Genetics of Complex Traits, Peninsula College of Medicine and Dentistry, Exeter, UK; 2) Broad Institute of MIT and Harvard, Cambridge, USA.

Recent genome-wide association studies have identified 43 independent loci where common variation influences human height. The identified loci explain only around 5% of the population variation of height, which is up to 90% heritable. These studies were based on additive, gender-combined analyses. However, final adult height is highly dichotomized by gender, and it is one of the polygenic traits most likely to have gender-specific SNP effects. In this study, we assessed the importance of gender-specific and non-additive effects in a height genome-wide association study.

As part of the GIANT consortium we meta-analysed ~2.4 million autosomal, directly genotyped or imputed, SNPs against Z-score (age-adjusted) height in 33000 (50% male) individuals from 13 studies. We performed gender-specific analyses under additive, dominant and recessive models. For the additive model, we tested if there was a differential effect between males and females.

Overall, there was no strong evidence for gender-specific effects, as the most highly gender-differentiated SNP had a gender difference $P=1x10^{-6}$, no better than expected by chance based on the number of tests performed. Similarly, there were no notable gender-specific associations when we limited the analysis to SNPs with overall $P<0.0001$ (best gender difference $P=5x10^{-3}$). When we looked for gender differences in SNPs that had $P<5x10^{-7}$ in either males or females, we found one potential gender-specific association (females $P=2x10^{-7}$; males $P=0.3$; difference $P=8x10^{-6}$). The associated SNP is in the *SYNE1* gene region, mutations of which cause recessively inherited cerebellar ataxia. Preliminary data from the recessive and dominant models have not identified any novel height associated loci.

In conclusion, gender-specific and non-additive effects appear to be of limited importance for genome-wide association studies of height, a model complex trait.

Principal Component Analysis and Hierarchical Clustering in 500K and 6.0 Genome Wide Association Datasets.
T. Drgon, C. Johnson, G. R. Uhl Dept Molecular Neurobiology, NIDA-IRP/NIH, Baltimore, MD.

Genome wide association (GWA) is a method of choice for elucidating polygenic influences in complex genetic disorders. However, the large numbers of repeated comparisons characteristic of GWA and the small anticipated sizes of the case/control allele frequency differences that are likely to be found in polygenic disorders motivate use of a variety of techniques for evaluating association signals. Principal Component Analysis (PCA) and Hierarchical Clustering (HC) array the variance found in multidimensional GWA datasets in matrices based on covariance (PCA) or geometric distance (HC). Variance components identified using these methods can be tested against the null hypothesis that no such component (or at least no component that explains certain threshold amount of the overall variance) can distinguish cases from controls using methods that are a priori agnostic to the structure in the data. We have used these approaches to analyze SNP and copy number variant (CNV) data from 500k and 6.0 array sets from samples of substance dependent and control individuals of European-American, African American and Asian origin studied as pools of DNA from 20 individuals. In these samples, both SNP and CNV datasets identified principal components that appear to capture much of the variance attributable to 1) ethnicity and 2) substance dependence. Using data from candidate genes, HC identifies significant clustering of a) pools of different ethnicities and of b) abuser and control pools within one ethnicity. We test the significance of such findings using permutation and Monte Carlo methods. These top down approaches provide assurance of overall genetic differences between case and control groups, and remind us of the modest sizes of these differences in comparison to racial/ethnic differences.

Familial longevity is not dependent of disease risk alleles identified in recent genome-wide association studies. *M. Beekman[1], B. T. Heijmans[1], C. Nederstigt[1], R. Van der Breggen[1], N. Lakenberg[1], J. J. Houwing-Duistermaat[2], R. G. J. Westendorp[3], P. E. Slagboom[1]* 1) Molecular Epidemiology, LUMC, Leiden, Netherlands; 2) Dept of Medical Statistics and Bioinformatics, LUMC, Leiden, Netherlands; 3) Dept of Gerontology and Geriatrics, LUMC, Leiden, Netherlands.

Ageing is the main risk factor for the major common human diseases that contribute to human mortality such as cardiovascular disease, cancer and type 2 diabetes. Centenarians show delayed ageing and among their offspring the prevalence of these age-related diseases is markedly decreased. Although partly heritable, genetic variation underlying familial longevity is as yet unknown. Long-lived families could by chance carry very few disease risk alleles, leading to their extreme survival. Alternatively, they may carry protective alleles, diminishing the effect of risk alleles. The first hypothesis can now be tested, using the robust associations with common age-related diseases published in the recent wave of genome-wide association studies (GWAs). We reviewed GWAs until May 31th 2008 on cardiovascular disease, cancer and type 2 diabetes. We selected 26 associated loci ($p<10^{-4}$) showing at least replication in one study or a second disease. At least one top SNP in each locus was tested for association with familial longevity in the Leiden Longevity Study, consisting of 959 long-lived siblings (mean age 93 yrs), 1765 of their offspring and the 758 partners of the offspring serving as population controls. None of the 26 loci from recent GWAs was associated with familial longevity. The estimated odds ratios were closer to 1 than in the published GWAs and the lowest observed p-value without adjustment for multiple testing was 0.055 (rs6922269). Next, we tested whether the total number of risk alleles was different between long-lived siblings and the controls, but this was not the case (p=0.256). These results indicate that long-lived families from the Leiden Longevity Study achieve high ages irrespective of the presence of the latest published common risk alleles. The deleterious effect of these alleles may be too small to affect mortality, or alternatively, longevity families may be protected against their effect.

Interstitial Deletion 6q25.2-q25.3 - A Novel Syndrome Associated With Microcephaly, Developmental Delay, Dysmorphic Features And Hearing Loss. *SC. Sreenath Nagamani[1], A. Erez[1], C. Eng[1], Z. Ou[1], C. Chinault[1], L. Workman[4], J. Coldwell[5], P. Stankiewicz[1], A. Patel[1], JR. Lupski[1,2,3], SW. Cheung[1]* 1) Dept Molec & Human Gen, Baylor Col Med, Houston, TX; 2) Dept of Pediatrics, Baylor College of Medicine, Houston, TX; 3) Texas Childrens Hospital, Houston, TX; 4) Sutter Medical Center, Sacramento, CA; 5) Children's Medical Center, Tulsa, OK.

Interstitial deletions of 6q are rare. We characterized the size, extent and genomic content of four interstitial deletions involving 6q25. Using a clinical targeted BAC or oligonucleotide array-CGH (Agilent, 44K), we identified submicroscopic deletions in 6q25.2-q25.3 in two patients and confirmed the cytogenetically visible 6q deletions in two patients. Genomic DNA from these patients was subsequently analyzed using an Agilent 244K oligonucleotide array. The deletions varied from 3.77 Mb to 13.81 Mb. All four patients had a common deleted segment in 6q25.2-q25.3, with the smallest region of overlap (SRO) spanning 3.52 Mb and containing fifteen genes mapping from TIAM1 to TULP4. As there was grouping of the proximal breakpoints in three of our patients in 6q25.2, we suggest that an underlying genomic architecture (e.g. palindrome or cruciform structures) important to the rearrangement is present in the vicinity of the deleted regions. Common clinical features seen in all four patients included microcephaly, developmental delay, dysmorphic features such as plagiocephaly, hypertelorism, abnormal nasal root, posteriorly rotated auricles and hearing loss. Hearing loss was of sensorineural type in three patients and conductive in one. Agenesis of corpus callosum was seen in two of the three patients who had imaging studies. We hypothesize that a subset of genes in the SRO are dosage sensitive and that their haploinsufficieny impairs normal development of the brain and hearing. We recommend that MRI of the brain and a formal hearing evaluation be done as a part of clinical assessment in patients with interstitial 6q25 deletions.

Family based association study for bipolar affective disorder identifies a candidate region on chromosome 1p31.3. *R. Secolin[1], C. E. M. Banzato[2], M. C. M. Oliveira[2], M. F. R. Bittar[1], P. Dalgalarrondo[2], I. Lopes-Cendes[1]* 1) Dept Medical Genetics, Univ Campinas - UNICAMP, Campinas, SP, Brazil; 2) Dept Medical Psychology and Psychiatry, Univ Campinas - UNICAMP, Campinas, SP, Brazil.

Bipolar affective disorder (BPAD) is a common psychiatric illness, with a prevalence of 0.8-2.6% in the general population. Clinical features include episodes of mania or hypomania, interspersed with periods of depression. Genetic factors are known to contribute to the etiology of BPAD. A recent large genome-wide population based association study performed in the British population identified 21 candidate loci across the genome. However, in order to confirm and strength these findings, further replication studies in different populations are needed. In the present study we aimed to evaluate the candidate loci previously reported using a family-based association approach. We evaluated 74 pedigrees with BPAD, with a total of 411 individuals, including 95 patients who fulfilled clinical criteria for BPAD according to ICD-10. We genotyped SNPs surrounding the candidate loci using real-time PCR by ABI 7500, TaqMan System (Applied Biosystems). We used TDT POWER CALCULATOR program in order to verify statistical power of our sample. Genotype data were processed by the LINKGEN program, which also estimates minor allele frequency (MAF). Mendelian inconsistencies and Hardy-Weinberg Equilibrium (HWE) were evaluated by PEDCHECK and HAPLOVIEW softwares, respectively. Family-based association analysis was performed by the UNPHASED software. Our sample has statistical power higher then 80% to detect association. To date, seven SNPs were analyzed and presented MAF 0.05. We found no Mendelian inconsistencies and HWE 0.001 for all SNPs genotyped. Only one single SNP (rs2989476) showed statistically significant association with BPAD (p 0.05). In conclusion, we demonstrated that SNP rs2989476, located on chromosome 1p31.3, is associated with the disease phenotype in our BAPD pedigrees. Additional SNPs are been genotyped in the candidate region in order to estimate haplotype association and to identify the putative causal variant associated with BPAD on chromosome 1p31.3. Supported by FAPESP.

Does raised adiposity increase blood pressure? The use of FTO and Mendelian randomisation in a large cohort study of causality. *N. Timpson[1], G. Davey Smith[1], J. Zacho[2], R. Harbord[1], A. Tybjærg-Hansen[2], B. Nordestgaard[2]* 1) CAiTE Ctr/Social Medicine, Univ of Bristol, Bristol, United Kingdom; 2) Herlev University Hospital, Denmark.

High body mass index (BMI) is associated with blood pressure (BP) and cardiovascular disease risk. If this association is causal, elevated BMI is a key target for intervention for BP reduction. Given the confirmed relationship between FTO (rs9939609) and BMI, we ultilised instrumental variable methods to estimate the strength and direction of the unconfounded association between BMI and BP. Within the Copenhagen General Population Study (n=37033), the correlation coefficients between systolic/diastolic BP and BMI were 0.21 and 0.24 (p=<0.001) respectively. In those not taking anti-hypertensive treatment where average BMI was 26.22kg/m2 (SD 4.27), the relationship between tertile of BMI and standardised BP was 0.17SD(0.16, 0.19) for systolic and 0.23SD(0.22, 0.24) for diastolic (p=<0.001) BP when adjusting for age, income, education, smoking and drinking. Using the FTO locus as an instrument for BMI in efforts to avoid confounding/reverse causation, equivalent estimates were revised to 0.36SD(0.18, 0.55) systolic and 0.27SD(0.07, 0.46) diastolic (p<0.001, 0.007) indicating that for each tertile of BMI, we predict an approximate causal change in BP of 1.5 and 1.2mmHg respectively. First stage F statistics confirm the strength of rs9939609 as an instrument for fat mass and a test for difference between observational and instrumental analyses showed there to be no consistent disparity between estimates (Wu-Hausman p>0.05). In a large, cross sectional, cohort of Europeans, a Mendelian randomisation approach yielded strong evidence for a causal relationship between BMI and BP as implicated by observational studies. Furthermore, estimates of life long exposure to elevated fat mass (derived from rs9939609), if anything, suggest a larger effect on blood pressure than basic analyses. These observations confirm BMI as an important target for therapeutic intervention aimed at lowering BP in concert with pharmaceutical approaches.

Aberrant activation of Rho GTPases in Smith-Lemli-Opitz syndrome: Neurodevelopmental and clinical implications. *X. Jiang[1], C. A. Wassif[1], L. Song[1], P. S. Backlund[2], A. L. Yergey[2], Z. Li[3], F. D. Porter[1]* 1) PDGEN, NICHD, NIH, DHHS, Bethesda, MD; 2) OSD, NICHD, NIH, DHHS, Bethesda, MD; 3) GCAP, NIMH, NIH, DHHS, Bethesda, MD.

Smith-Lemli-Opitz syndrome (SLOS) is a malformation syndrome with neurocognitive deficits due to mutations of *DHCR7* that impair synthesis of cholesterol. To investigate the pathological processes underlying the neurocognitive deficits found in SLOS, we performed proteomic analysis comparing protein expression in E18.5 *Dhcr7*+/+ and *Dhcr7*-/- mouse brains. Combining 2D-PAGE analysis and mass spectrometry techniques we identified 52 proteins with differential expression. One of these proteins was cofilin. Cofilin regulates depolymerization of actin, and plays a major role in regulation of cytoskeletal structure. Western blot analysis showed that the apparent differential expression of cofilin was due to increased phosphorylation. Phosphorylation of cofilin alters its activity, and is regulated by Rho GTPases through both the Rho-Rock-Limk-Cofilin and Rac/Cdc42-Pak-Limk-Cofilin pathways. Rho GTPases cycle between inactive GDP-bound and active GTP-bound states. We used GST-pull-down assays to demonstrate increased activation of RhoA, Rac1, and Cdc42 in *Dhcr7-/-* brains. Consistent with increased activation of Rho GTPases, we also found increased phosphorylation (activation) of Limk and Pak in mutant brain tissue. This explains altered cofilin phosphorylation. Rho GTPases play a central role in the regulation of axonal and dendrite growth. Altered Rho/Rac signaling impairs normal dendrite and axon formation, and mutations in genes encoding regulators and effectors of the Rho GTPases have been found to underlie other human mental retardation syndromes. Thus, we hypothesized that aberrant activation of Rho/Rac could have functional consequences for dendrite and axonal growth. *In vitro* functional analysis of *Dhcr7-/-* hippocampal neurons demonstrated significant abnormalities of both axon and dendrite growth. Thus, developmental abnormalities of neuronal process formation may contribute to the neurocognitive deficits found in SLOS, and may represent a potential target for therapeutic intervention.

Identification of a putative association between a common variant in the Thyroid stimulating hormone receptor (TSHR) gene, known to predispose to autoimmune thyroid disease, and preterm birth. *T. M. Frayling[1], R. M. Freathy[1], D. Velez[2], J. Bartlett[2], S. Fortunato[3], S. M. Ring[4], B. Shields[1], A. T. Hattersley[1], S. M. Williams[2], G. Davey Smith[4], R. Menon[3], C. Relton[5]* 1) Peninsula Medical School, Exeter, UK; 2) Vanderbilt University, Nashville, TN, USA; 3) The Perinatal Research Center, Nashville, USA; 4) Bristol University, UK; 5) Newcastle University, UK.

Preterm birth, defined as a gestational age <37 weeks, is one of the leading causes of neonatal morbidity and mortality. There is strong evidence that preterm birth has a genetic component, but the genes involved, and the extent to which offspring and maternal genotypes play a role, are not known. We performed the largest preterm birth genetic association study to date, consisting of 729 offspring cases, 593 maternal cases, 920 offspring controls and 772 maternal controls. We analysed ~930 SNPs in 134 candidate genes from pathways thought to play key roles in preterm birth. The most strongly associated offspring genotypes were those in the thyroid stimulating hormone receptor (TSHR) gene. A cluster of SNPs from across the TSHR gene, were associated with preterm birth. The strongest association was with rs2075173, with an odds ratio of 1.38 (95%CIs:1.11-1.72; p=0.003). This and other SNPs representing the signal were in linkage disequilibrium (r2=0.12 to 0.7) with a non-synonymous SNP, S248R, known to predispose to autoimmune thyroid disease. The strongest maternal association was with rs3211719 in the Factor VII gene, with an odds ratios of 1.33 (95%CIs:1.11-1.59; p=0.002). The most common variant known to alter the risk of thrombosis, R353Q, in Factor VII, was not associated with pre-term birth (odds ratios of 0.97 (95%CIs:0.75-1.26; p=0.19) using an r2=0.93 proxy. SNPs in another gene involved in the coagulation-complement pathway, Plasminogen activator tissue (PLAT), were the fourth strongest maternal signal (rs879293, p=0.006). Thyroid function is known to be altered in preterm infants. Our results need to be followed up in additional samples but provide the first evidence that altered thyroid function may be causal to preterm birth.

Assessing Public and Biobank Participant Attitudes toward Data Sharing for Genome Wide Association Studies: A Community Consultation Approach. *A. A. Lemke[1], W. A. Wolf[1], J. Hebert-Beirne[2], R. L. Chisholm[1], M. E. Smith[1]*
1) Northwestern University, Chicago, IL; 2) Women's Health Foundation, Chicago, IL.

Recent policies requiring sharing of research data on participants included in genome-wide association studies have not been fully assessed. This study utilizes a community consultation process to elicit feedback and opinions from participants in the NUgene, a hospital-based biorepository, and a diverse group of community members. The study objectives are: 1) to obtain in participants own words their attitudes toward collecting, analyzing and sharing genetic research data; 2) to compare attitudes between biorepository participants and the public; and 3) to offer recommendations for the consent process and sharing of genetic research data. Forty-nine individuals participated in six focus groups; 21 in three NUgene biorepository focus groups and 28 in three public focus groups. All participants completed a brief background information survey. In the NUgene focus groups, 67% of participants were women, 95% had some college education or more, and the majority (80%) of participants were Caucasian (20% African-American). In the public focus groups, 75% of participants were women, 75% had some college education or more, 29% were Hispanic, and the majority (46%) were African-American (29% Caucasian, 21% other). More than half (56%) of the NUgene focus group participants were somewhat or very concerned about the confidentiality and privacy of medical information compared to 46% of the public group participants. Overall, approximately 75% of focus group participants were neutral or somewhat trusting of medical research. Preliminary themes identified in both focus group types include: barriers and incentives toward participating in genetic research; conditions necessary to consent to participation in genetic research; concerns about the privacy of individual research data, and apprehension regarding the role of institutions and the government in the protection of genetic research data. Findings from this research will be used to inform key professional groups regarding concerns and potential changes needed in the consent and genetic research data sharing process.

Nuclear architecture alterations in a patient with a novel heterozygous missense LMNA mutation. *F. Gullotta[1], D. Postorivo[1], F. Lombardi[2], S. Latini[2], A. Vielle-Canonge[2], K. Petrilli[1], A. M. Nardone[1], M. R. D'Apice[1,2], M. Bertoli[2], M. D'Adamo[3], P. Sbraccia[3], S. Servidei[4], G. Novelli[1,2,5]* 1) Department of Medical Genetics, A.O.U. Policlinico Tor Vergata, Rome, Italy; 2) Department of Biopathology and Diagnostic Imaging, University of Rome Tor Vergata, Rome, Italy; 3) Department of Internal Medicine University of Rome Tor Vergata, Rome, Italy; 4) Department of Neuroscience, Catholic University, Rome, Italy; 5) University of Arkansas for Medical Sciences, Little Rock, Arkansas.

We report a 27-yr-old Italian girl with a complex disorder characterized by extreme leanness, severe insulin resistance, mixed calcific valvulopathy involving both mitral and aortic valves, slight facial dismorphism, low femoral bone mass. LMNA sequencing evidenced a novel heterozygous missense mutation in exon 4 that replaces well-conserved residue glutamic acid at position 262 to lysine (p.E262K, c.784 GA). Her parents, not consanguineous, were negative for the same mutation, suggesting its de novo origin. Genetic screening of additional genes mutated in laminophatic or lipodistrophyc disorders (ZMPSTE24, PPAR, BSCL2) showed a wild type sequence. In order to investigate the effects of this mutation on the nuclear architecture, we examined the nucleus morphology, the unprocessed precursor pre-lamin A protein, and the chromatin organization through the study of heterochromatin protein trimethyl histone H3 (Lys9). Patients fibroblasts showed numerous and eterogeneous nuclear shape alterations and pre-lamin A accumulation at the nuclear rim but also, in a lower percentage of cells, in intranuclear foci. Heterochromatin disorganization was observed resulting in loss of trimethyl histone H3 (Lys9) clustering. The three markers selected to check the cellular phenotype in our patient have already been described altered in fibroblasts of patients whit Mandibuloacral dysplasia type A [MADA; OMIM # 248370], a rare autosomal recessive disorder caused by an homozygous missense mutation (p.R527H) in C-terminal tail domain of LMNA gene.

Reconsidering reproductive benefit: A systematic review of guidelines on preconception, prenatal and newborn screening. *Y. Bombard[1], F. A. Miller[1], R. Hayeems[1], D. Avard[2], B. M. Knoppers[2]* 1) U. Toronto; 2) U. Montréal.

Introduction: Traditionally newborn screening (NBS) aimed to reduce morbidity and mortality among screened infants. Its mandatory nature was justified by the primary benefit of improved clinical outcomes in affected babies. Expanded NBS programs identify conditions for which treatment is not established as well as benign carrier states or variants of uncertain clinical significance. In consequence, expansion is accompanied by debates about the nature of benefit and the appropriateness of a mandatory program. Chief among secondary benefits is the opportunity to inform parents and infants of future reproductive risks (RR). However, in the absence of clinical benefit, pursuit of RR information through NBS requires consideration. Although their historical contexts and value systems differ, preconceptual screening (PCS) & prenatal screening (PNS) programs may be informative paradigms for considering how to pursue reproductive benefit through NBS. **Purpose:** To explore how PCS & PNS guidance can inform NBS guidelines, we investigated: 1.Whether the generation of RR information is pursued as a benefit of screening; 2.How this benefit is to be realized (e.g. voluntarism). **Methods:** All available English guidelines on NBS, PCS & PNS from inter-, national and regional governmental and non-governmental health organizations were retrieved using HUMGEN and other sources. **Results:** All PCS & PNS guidelines pursue the generation of RR information as a benefit of screening, and a majority requires that individuals be given the choice to learn of their RR. By contrast, few NBS guidelines identify the generation of RR information as a benefit and for most, this is a secondary goal. While many NBS guidelines stress the importance of educating parents about NBS, few require consent. **Discussion:** Insights gleaned from PCS & PNS guidance suggest that mandatory NBS may be ethically untenable in its expanded form. If what constitutes benefit widens to include ascertaining RR information some form of voluntarism becomes a fundamental condition. Alternatively, achieving reproductive benefit in the absence of clinical benefit may be better pursued through antenatal screening programs.

A comprehensive evaluation of the Interferon Induced with helicase C domain 1 gene (IFIH1) reveals further evidence for association in families with type 1 diabetes. *K. Keene, S. Onengut-Gumuscu, P. Concannon* Center for Public Health Genomics, University of Virginia, Charlottesville, VA.

Type 1 diabetes (T1D) is a complex autoimmune disease that involves both genetic and environmental risk factors. Genome wide association studies have recently provided a new list of potential T1D predisposing genes including the Interferon Induced with helicase C domain 1 gene (IFIH1), a putative RNA helicase that has been implicated in innate immune defense against viruses. A nonsynonymous single nucleotide polymorphism (SNP), rs1990760, has been shown to be associated with T1D in case-control and family studies. However, the subsequent association with rs3788964 and relatively low correlation (r^2= 0.26) between the two SNPs suggests that multiple variants within IFIH1 may contribute to T1D risk. In order to fully understand the functional role of IFIH1 in T1D susceptibility, it is imperative that the causal variants are identified and characterized. Using sequencing and fine-mapping approaches, we aimed to identify and subsequently perform tests of association for variants within the IFIH1 gene region. We re-sequenced all 16 exons and intron/exon boundaries of IFIH1 in 48 individuals, confirming 16 previously identified SNPs and three novel SNPs. Thirteen SNPs were genotyped, using the Eclipse genotyping platform, in 382 multiplex T1D families from the Type 1 Diabetes Genetics Consortium. Single SNP and haplotype association was performed using Family Based Association Tests (FBAT). Eight SNPs and three haplotypes provided evidence for association (P< 0.05) with T1D. The most significant single SNP associations were observed with rs12479043 (P= 0.00516), rs1990760 (P= 0.00569), rs3747517 (P= 0.00756), and rs3747518 (P= 0.00796), while rs3788964 provided modest evidence of association (P= 0.0404). One common risk haplotype (45% frequency; Z= 2.624; P= 0.00868), and two uncommon protective haplotypes (7.6% frequency; Z= -2.115; P= 0.0344 and 1.4% frequency; Z= -2.364; P= 0.0181) were significantly associated with T1D. These results suggest that the association between IFIH1 and T1D may be more haplotype-based rather than specific to one particular SNP.

Routine application of an extended prenatal diagnosis panel (EPP) for the targeted detection of known microdeletion syndromes and subtelomeric deletions/duplications by MLPA analysis. *C. Pangalos[1,2], B. Hagnefelt[2], S. Karapanou[2], C. Konialis[2]* 1) Clinical Genetics, Diagnostic Genetic Ctr, Athens, Greece; 2) Molecular Genetics, Diagnostic Genetic Ctr, Athens, Greece.

There are a relatively large number of known genetic syndromes, as well as newly discovered ones, now described as genomic disorders, involving gain or loss of genetic material at a level not detectable by routine karyotype analysis. Although individually rare, these genetic syndromes may occur collectively in ~1 in 1,200 live births. Subtelomeric rearrangements are recognized as the underlying cause of idiopathic mental retardation, with or without congenital abnormalities, in 5-12% of patients studied. Prenatal ultrasound examination and karyotype analysis will typically fail to detect most, if not all, of the above anomalies in the fetus. We describe the application of a multiplex ligation-dependent probe amplification (MLPA) approach for the simultaneous targeted detection of 19 known microdeletion syndromes (1p-deletion syndrome, Williams-Beuren, Smith Magenis, Miller-Dieker, DiGeorge, VCFS, Cat Eye, Prader-Willi/Angelman, Sotos, Wolf-Hishhorn, Cri du Chat, Langer-Giedon, WAGR, 2p16, 3q29, 9q22, 15q24,17q21, 22q13) as well as subtelomeric copy number assessment for all chromosomes, as an extension to routine prenatal karyotype analysis in a diagnostic service laboratory. Using MLPA probe sets P064, P096, P245, P036D and P070 (MRC-Holland, The Netherlands) and analysis with GeneMarker software (Softgenetics LLC), the detection sensitivity for the selected syndromes is 50-98%, coupled to reliable detection of all subtelomeric deletions/duplications. This panel has been applied in 520 random chorionic villi and amniotic fluid samples referred for routine prenatal karyotype analysis, with or without U/S findings. We identified one unsuspected case for DiGeorge syndrome in a 17 week fetus, referred only because of advanced maternal age, and the result was further confirmed by FISH analysis. The protocol is relatively cheap, robust, rapid, targeted and sensitive, while genetic counseling in case of abnormal results is fairly uncomplicated compared to abnormal arrayCGH results in prenatal samples.

Capacity Building for the Transfer of Genetic/Genomic Knowledge into Practice and Prevention: The CAPABILITY International Collaborative Network. *I. Nippert[1], U. Kristoffersson[2], J. Schmidtke[3], A. Kent[4], A. Christianson[5], R. Raouf[6], C. Barreiro[7]* 1) Women's Hlth Res, Univ Muenster, Muenster, Germany; 2) Dept Clin Gen, Univ Hosp Lund, Lund, Sweden; 3) Inst Hum Gen, MHH, Hannover, Germany; 4) GIG, London, UK; 5) Div Hum Gen, Univ Witwatersrand, Johannesburg, South Africa; 6) Children with special needs Dep, Ministry of Health, Cairo, Egypt; 7) Hospital de Pediatria Garrahan, SAMIC, Buenos Aires, Argentina.

The number of genetic tests is growing each year and increasing knowledge about gene-disease associations will lead to new opportunities to apply genetic/genomic knowledge in practice and prevention. Before genetic tests are introduced into general practice the benefits of their use must be evaluated. Worldwide, health care systems are facing the same challenges: 1) The need to develop an evidence-based evaluation process for genetic tests or other applications of genomic knowledge in transition from research into practice. 2) The need for capacity building to enable health care systems to make effective use of genetic/genomic applications with proven clinical utility. CAPABILITY (http://www.capabilitynet.eu) is a 3-year model project developed by the European Network of Excellence: Genetic Testing in Europe - Network for test development, harmonization, validation and standardization of services (EuroGentest) (http://www.eurogentest.org) and by leading experts from emerging economies: Argentina, Egypt and South Africa, the latter being currently engaged in major development projects to integrate genetic services in primary care and prevention in their countries. CAPABILITY methods: develop an analytic framework for evidence-based genetic test evaluation identify priorities for capacity building by a systematic needs assessment survey and validate the project's approach by means of a demonstration project. CAPABILITY's overall objectives are to contribute to the efforts to establish and sustain a worldwide harmonisation process for quality standards for the integration of genetic test/genomic knowledge applications into practice and prevention and to serve as a model project for successful, sustainable international collaboration.

Identification of a novel mutation homozygous mutation in the SIL1 gene in Marinesco-Sjögren syndrome (MSS). *G. Annesi[1], C. Cerami[2], C. Cupidi[2], P. Tarantino[1,4], E. V. De Marco[1], F. Annesi[1], V. Lo Re[2], E. Mannarino[1,4], G. Provenzano[1,4], A. Quattrone[1,3]* 1) Inst Neurological Sci, National Res Council, Cosenza, Italy; 2) Dept. Clinical Neurosciences, University of Palermo, Palermo, Italy; 3) Institute of Neurology, University Magna Graecia, Catanzaro, Italy; 4) Departement of Neurosciences, Psychiatry and Anaesthesiology University of Messina, Messina-Italy.

MSS is an autosomal recessive disorder, characterised by cataracts, ataxia, and growth and mental retardation. Chronic myopaty is a common feature, and peripheral neuropathy has also been described. Recently, Anthonen et al showed the linkage of the MSS phenotype to 5q31 chromosome in a Finnish family, and identified mutations of the SIL1 gene in all investigated MSS patients. Senderek et al, using homozygosity mapping in three small consanguineous families with typical MSS, narrowed a critical region on 5q31. In this region the authors identified 9 distinct mutations in SIL1 gene in individuals from eight small families with MSS . In this study we analyzed the SIL1 gene in a small pedigree from Sicily with one patient clinically definite MSS . He shows classical MSS with psychomotor delay, hypergonadotrophic hypogonadism and distinct cerebellar symptoms.He showed poor vision and the early infantile period, which was diagnosed as cataract and was surgically removed later on. Brain MRI of the patient showed marked cerebellar atrophy. We sequenced all 10 exons of the BAP/SIL1 gene in our MSS pedigree (heterozygote parent and affected individual). Sequencing of the SIL1 gene revealed a novel homozygous mutation in exon 10 (1207delG) and a new homozygous mutation in the same exon (1209delC). Both mutations cosegregate with the disease and were detected in a heterozygous state in the patientmother and father. We did not find the same mutation in 100 chromosomes from healthy individuals from Sicily. This mutation causes a frameshift in translation and premature termination of SIL1 protein (V403fsX427), deleting 24 amino acids from the end of the protein. Our study strengthen the previous findings of loss-of-function mutations in SIL1 being the major cause of MSS.

Identifying Gene X Gene Interactions for phenotypes in Genome-wide Association Studies. *J. Wu, K. Roeder*
Statistics, Carnegie Mellon University, Pittsburgh, PA.

More and more studies have confirmed that interactive effects, or epistasis, of two or more genetic variants plays a key role in the understanding of liability to complex disease. We explore a procedure called screen and clean by Roeder and Wasserman (2008) for identifying liability loci, including interactions, in genome-wide association studies. Instead of testing each locus marginally, we identify the most promising SNPs and interactions simultaneously using the lasso procedure (Effron et. al 2004). Because the class of models including all interactions is too large to be practical we approach the problem using a varying dictionary. In the first step the lasso dictionary includes only main effects. The most promising SNPs are identified using a screening procedure designed to identify SNPs involved in interactions as well as main effects. Next the lasso dictionary is adjusted to include these main effects and the corresponding interaction terms. Again, promising terms are identified using a lasso screening procedure. Only those SNPs identified by the stage 1 screening process are genotyped in stage 2. Significant terms are identified through the cleaning process. We applied our procedure on both simulated data and data simulated with linkage disequilibrium and allele frequencies identical to a panel of SNPs from a candidate gene study. We compared our results with marginal two stage tests such as Lin (2006) and LeBlanc and Kooperberg (2008). Our method has comparable or higher power than the marginal two stage tests. Moreover, our method provides an accurate control of the type I error and false discovery rate when the markers are correlated. The R code is available at www.stat.cmu/~jwu/screenNclean/.

Effect of Fibrinolysis on Bronchopulmonary Dysplasia in Newborns. *H. Verdi[1], FB. Atac[1], D. Ince[2], A. Taneri[1], E. Sezgin[1], AC. Yazici[3], A. Tarcan[2], N. Ozbek[2]* 1) Dept Medical Biol and Genetics,Baskent Univ, Ankara, Turkey; 2) Dept Pediatric, Baskent Univ,Ankara,Turkey; 3) Dept Biostatistics, Baskent Univ, Ankara, Turkey.

Bronchopulmonary dysplasia (BPD) is a chronic disease that affects premature babies and contributes to their morbidity and mortality. A new BPD has been characterized in preterm infants that may begin in utero, then progress postnatally, resulting in arrested lung development and alveolar hypoplasia. It is defined as oxygen therapy requirement at the age of 28 days for premature infants. The severity of BPD is defined in infants born at <32nd gestational week based on their oxygen requirement on the 36th gestational week or at the time of discharge to home, and in infants born on >32nd gestational week based on their oxygen requirement on the 56th postnatal day or at the time of discharge to home. Factor XIII (F13) Val34Leu, angiotensin-converting enzyme insertion/deletion (ACE I/D) and the plasminogen activator inhibitor-1 gene (SERPINE1, also known as PAI1) 4G/5G are the three hypofibrinolytic gene variants that may enroll a cumulative effect in the development of BPD phenotype. To test this hypothesis a total of 192 newborn infants (98 BPD cases and 94 controls) were included. The obtained genomic DNA was used as a template for the investigation of F13 Val34Leu, ACE I/D and PAI-1 4G/5G genotypes. The preliminary results of our study indicates that ACE D, SERPINE1 4G and F13 34Leu alleles did not have a cumulative effect on the development BPD in our population.

Overexpression of microRNA-206 in the skeletal muscle from myotonic dystrophy type 1 patients. *S. Gambardella[1], F. Rinaldi[1], A. Viola[1], L. Vergani[2], E. Loro[2], V. Romeo[2], C. Angelini[2], G. Novelli[1], A. Botta[1]* 1) Dept Biopathology, Tor Vergata Univ, Rome, Italy; 2) Dept of Neurology, University of Padoa, Padoa, Italy.

MicroRNAs are highly conserved, noncoding RNAs involved in post-transcriptional gene silencing. They have been shown to participate in a wide range of biological processes, including myogenesis and muscle regeneration. The goal of this study is to test the hypothesis that myo-miRs (myo=muscle+miR=miRNA) expression is altered in muscle from patients affected by myotonic dystrophy type 1 (DM1), the most frequently inherited neuromuscular disease in adults. To this aim, we have profiled the expression miR-133 (miR-133a-1, miR-133a-2, miR-133b), miR-1 (miR-1-1, miR-1-2), miR-181 (miR-181a, miR-181b, miR-181c) and miR-206, that are specifically induced during myogenesis in the heart and skeletal tissues. QRT-PCR experiments have been performed on RNA from vastus lateralis biopsies of DM1 patients (n=7) and control subjects (n=4). Only miR-206 showed an over-expression in 5 of 7 DM1 patients (threshold=1.5, fold change between 1.68 and 13.51, average= 5.02) compared to control group. This result has been further confirmed by Northern blot analysis (4.2-fold overexpression). The misregulation of miR-206 in DM1 is consistent with what observed in the affected diaphragm of mdx mouse (an animal model of Duchenne muscular dystrophy). Since the vastus lateralis from DM1 patients exhibits all the pathological hallmarks of a dystrophic tissue, miR-206 may contribute to the chronic course of both muscular dystrophies. Further investigation are in progress to characterize the protein level of miR-206 targets and to understand if the excess of this miR colocalized with the ribonuclear foci observed in DM1 cells. This study is supported by Telethon (GGP07250).

Multivariate genetic analysis of specific language impairment (SLI) in extended pedigrees: The relationship between reading and language measures on 13q21. *T. R. Simmons[1], P. J. Weaver[1], T. Andrews-Bryant[1], J. F. Flax[2,3], P. Tallal[2], C. W. Bartlett[1], L. M. Brzustowicz[3]* 1) Battelle Center for Mathematical Medicine, The Research Institute at Nationwide Childrens Hospital, Columbus, OH; 2) Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark, NJ; 3) Department of Genetics, Rutgers University, Piscataway, NJ.

SLI is a heritable developmental language disorder occurring in absence of frank neurological or cognitive impairment. It is characterized by significant problems of comprehension and/or expression of spoken language. However, the difficulty defining SLI and the complex relationship between language and reading (in SLI) requires us to understand phenotypic and genetic heterogeneity in the context of the different phenotypic pathways to language impairment. Previously, we found compelling evidence for an SLI susceptibility locus using a reading impairment (RI) variable on 13q21-22 with a PPL of 53% (LOD=3.92). This finding was followed up with a replication PPL of 17% (LOD=2.62); joint analysis of both datasets shows strong evidence for linkage to 13q21 with a PPL of 96.9% (LOD=7.86). Despite the overwhelming evidence for a susceptibility allele in 13q21, prima fascia, it is unclear how reading impairment relates to either SLI or quantitative measures of language at this locus. In order to elucidate the relationship, we performed a joint analysis where persons affected with RI are left coded as affected while the remainder of the individuals retain their quantitative language scores in the analysis. Our model implicitly assumes that RI is at the low end of the language score distribution. The PPLs for this model were 65%, 37% and 15% using three different language measures. We also performed the complimentary linkage analysis where language impairment was analyzed with several quantitative reading measures (all PPLs < 5%). This dissociation is consistent with our hypothesis that, in our sample selected for language deficits, RI is due to either an underlying language impairment or both reading and language deficits are caused by the same (similar) underlying factors.

Parkin, Pink1 and DJ-1 heterozygous mutations in southern italian patients with early-onset parkinsonism. *F. Rocca[1,2], F. Annesi[1], E. V. De Marco[1], D. Civitelli[1], P. Tarantino[1,3], G. Provenzano[1,3], V. Scornaienchi[1], V. Greco[1], G. Annesi[1]* 1) Inst Neurological Sci, National Research Council, Cosenza, Italy; 2) Institute of Neurology, University Magna Graecia, Catanzaro; 3) Departement of Neurosciences, Psychiatry and Anaesthesiology University of Messina, Messina Italy.

Recessively inherited early-onset Parkinsonism (EOPD) has been associated with mutations in the PARKIN, PINK1 AND DJ1 genes. Screening patients cohorts has revealed that more than 50% of patients with mutations in PARKIN or PINK1 have only a single heterozygous mutation. To evaluate the frequency of heterozygous mutations in genes associated with autosomal recessive EOPD, we investigated 134 sporadic cases of EOPD from southern Italy. The genetic screening was performed by direct sequencing of the coding region of PARKIN, DJ1 and PINK1 genes. All exons of PARKIN and DJ1 genes were examined for gene dosage abnormalities in individual with single heterozygous mutations.Moreover in patients carrying of only one mutation in the PARKIN gene, we also investigated rearrangements in the promoter region. Screening identified single heterozygous mutations in 11 EOPD patients. Eight patients (5,9%) carried heterozygous mutations in PARKIN gene (five novel variants: Thr55Ile, Asp18Asn, Lys 32Thr, Asp86Asn, Pro37Pro and three described variants: Arg42Pro, Leu261Leu, Met192Leu). Two patients (1,4%) showed novel heterozygous mutations in DJ1 gene (g159C/G and Asp68Val). One patient (0,7%) carried a novel heterozygous mutation in PINK1 (R207Q). Novel variants were absent in 100 control chromosome. Gene dosage experiment failed to reveal rearrangements within PARKIN and DJ1 genes and in the PARKIN promoter. In our population the frequency of heterozygous mutations in PARKIN and DJ1 genes is similar to that reported by other authors, whereas heterozygous mutations in PINK1 gene are less frequent.The role of single heterozygous mutations in EOPD is difficult to interpret. Haploinsufficiency or a dominant negative effect of the mutant allele may account for the pathogenesis.

Genetic risk estimation in Crohns disease. *C. M. Lewis[1,2], C. G. Mathew[1]* 1) Medical & Molecular Genetics, King's College London, UK; 2) MRC SGDP Centre, Institute of Psychiatry, King's College London, UK.

Genome-wide association studies have enabled researchers to identify disease-gene associations, detecting many novel susceptibility genes for common, complex disorders. However, most variants confer only a small increase in risk and are of questionable value for prediction studies, identifying individuals who are at increased risk of disease. To explore the potential for population screening in Crohns disease (CD) we analyzed six confirmed susceptibility loci for CD, including NOD2. Risks were extracted from UK replication studies. Most SNP effects fitted a multiplicative model, with allele-specific relative risks (RR) ranging from 1.10 (ATG16L1; T300A) to 1.45 (IL23R; rs10889677). For NOD2, individuals were classified by the number of mutations carried, and showed a gene dosage effect (RR for 1 mutation: 2.2, for 2 mutations: 9.2). Assuming no gene-gene interaction, we estimated the relative risk of CD for each genotype combination across SNPs. The RR of CD in the highest risk genotype compared to the lowest risk genotype is >150. This commonly used comparison is inappropriate (although commonly used) as each of these genotypes is extremely rare. Using a baseline genotype whose prior, population risk (assumed to be 0.001) is equal to its posterior risk provides more appropriate (if less striking) relative risks. Then, individuals with genotypes in the top 10% of the risk distribution have relative risks of 1.7 - 45.0, and account for 26% of the CD cases in the population. Only 1% of the population has a RR > 4. Individuals with genotypes in the lowest risk decile are protected against CD, with relative risks of >0.28, and account for only 4.5% of CD cases. NOD2 provides the largest contribution to risk estimation, with other genes having a limited modifying effect. These results suggest that genetic risk profiles for CD will currently be of limited value for population screening, as only a small proportion of the population has substantially increased risk. Focusing tests on those with positive family history or presence of environmental risk factors will be more useful, and can be included in the risk modeling.

FBN1 gene screening in 586 probands reveals the minimal combination of clinical features to predict efficacy of mutation identification. *C. Stheneur[1,2], G. Collod-Beroud[3], L. Faivre[4], L. Gouya[2,5,6], B. Grandchamp[2], B. Chevallier[1,2,6], G. Jondeau[2], C. Boileau[2,5,6,7]* 1) Service de pédiatrie, APHP-Hôpital A.Pare, Boulogne, France; 2) Consultation multidisciplinaire Marfan, APHP-Hôpital Bichat, Paris, France; 3) INSERM U827 Montpellier, France; 4) CHRU Dijon Centre de genetique Dijon, France; 5) Laboratoire central de biochimie d'hormonologie et de genetique moleculaire, Boulogne, France; 6) Universite Versailles-SQY, Versailles, France; 7) INSERM U781, Paris France.

Mutations identified in the FBN1 gene have been associated with Marfan syndrome (MFS) and a wide range of overlapping disorders. Molecular analysis of the gene is performed in probands with classical MFS, to offer diagnosis for at-risk relatives and in children highly suspected of MFS. We screened the FBN1 gene in 586 probands who had been addressed for molecular diagnosis. We identified 354 mutations representing 196 missense mutations, 48 splice site alterations, 55 nonsense mutations, 14 insertions/duplications and 41 deletions. We found that the efficacy of FBN1 gene screening was high in classical MFS probands (75.4 %,); lower (62.9%) in those referred for incomplete MFS and only slight (14.3%) for patients referred as possible MFS but with no major diagnostic criterion (as described in the Ghent nosology). Recursive partitioning was used to build a regression tree to identify combinations of clinical features related to mutation detection rate. We found that the best predictor of the identification of a mutation in the FBN1 gene for incomplete MFS was the presence of features in at least three organ systems, combining one major and various minor criteria. Finally our data also show that our original recommendation (two systems involved with at least one with major criterion), represent the minimal criteria since, in probands not meeting these criteria the yield of mutation identification drastically falls. This recommendation should help clinicians and biologists in identifying probands with a high probability of carrying a FBN1 gene mutation, and thus optimize biological resources.

Family history of hepatoblastoma or hepatocellular carcinoma in children with liver disease in a genetic isolate: a clue for a recessive trait. *M. Girard[1,2], F. Lacaille[2], F. Sauvat[3], F. Jaubert[4], L. Brugières[5], I. Aerts[6], J.-L. Michel[7], V. Verkarre[4], L. Galmiche[4], M. Fabre[8], S. Lyonnet[1], A. Henrion Caude[1]* 1) Dept Genetics, Inserm U781, University Paris Descartes, Necker Hospital, Paris, France; 2) Dept Pediatric Gastoenterology, Necker Hospital, Paris, France; 3) Dept Pediatric surgery, Necker Hospital, Paris, France; 4) Dept Anatomopathology, Necker Hospital, Paris, France; 5) Dept Pediatric Oncology, Gustave Roussy Institute, Villejuif, France; 6) Dept Pediatric Oncology, Curie Institute, Paris, France; 7) Dept Surgery, Felix Guyon Hospital, Reunion Island, France; 8) Dept Pathology, Kremlin-Bicêtre Hospital, University Paris Sud-11, Le Kremlin-Bicêtre, France.

Here we report on the occurrence of either hepatoblastoma or hepatocellular carcinoma, together with chronic liver disease, in three patients, with onset between the neonatal period and 9 years of age. The liver disease was characterized by fibrosis and cholestasis. Extensive questioning of the family revealed no known cases of adenomatous polyposis. Along these lines, all known causes for chronic liver disease were ruled out for each of the three patients. Two of the children with hepatoblastoma and hepatocellular carcinoma were siblings, while the third hepatoblastoma patient was a second cousin. All patients originate from the Reunion Island, a population prone to founder effects. To the best of our knowledge, this is a first description of the occurrence of both hepatoblastoma and hepatocellular carcinoma in a single extended family. The common genetic background as well as the existence of a primitive liver disease in these related cases raise the possibility of a unique recessive trait predisposing to liver tumors in childhood. The results of a genome-wide linkage analysis currently performed will be presented, using the affected-only approach and considering several alternative underlying genetic models.

Adapting the Logical Basis of Tests for Hardy Weinberg Equilibrium. *K. Goddard[1], A. Ziegler[2], S. Wellek[3]* 1) Ctr Health Res, Kaiser Permanente Northwest, Portland, OR; 2) Institute of Medical Biometry and Statistics, University at Lübeck, Germany; 3) Division of Biostatistics, CIMH Mannheim/University of Heidelberg, Germany.

The standard goodness-of-fit (GOF) test to assess HWE is fundamentally flawed with respect to its logical basis, because it is tailored to establish departure from HWE. However, in the majority of applications, we aim to exclude the existence of gross violations of the equilibrium condition. We present a logically unflawed solution to this problem using equivalence testing, and show that the new method provides exact control of the type I error risk. The test is available in one- and two-sided versions, and we provide tools for exact power calculation. The power is 80% for a wide range of allele frequencies when the sample size exceeds 200. We illustrate the method using genotype distributions from 43 candidate gene studies and 2 genome-wide association studies. The conclusions of the GOF and equivalence tests are the same for 70% of the samples. There are two explanations for the discrepancies. First, when the sample size is small, the default behavior of the two tests leads to discordant conclusions in circumstances of low power. For the GOF test, tests with low power fail to reject HWE, and tend to be classified with tests indicating compatibility with HWE. For the equivalence test, tests with low power fail to demonstrate equivalence with HWE, and tend to be classified as indicating a lack of fit to HWE. In the observed studies with this type of discrepancy, the average sample size is 142 (84) for the discordant studies, and 212 (192) for the concordant studies. Second, when the sample size is large, statistically significant, but small, deviations from HWE are detected in the GOF test. We conclude equivalence with HWE using the equivalence test, because these small deviations are unimportant. The magnitude of the deviation from HWE for the discordant studies is about half of the deviation from HWE for the concordant studies (.11 .023 versus .19 .14, respectively). The new test provides more satisfactory assessment of HWE, especially for genome-wide association studies.

The Melatonin Receptor 1A Gene (MTNR1A) Is Associated With Kidney Stones. *R. Chimienti[1], A. Aloia[1], D. Rendina[2], A. Ciccodicola[1], P. Strazzullo[2], F. Gianfrancesco[1], G. Mossetti[2], T. Esposito[1]* 1) Institute of Genetics & Biophysics, Italian National Research Council, Naples, Italy; 2) Department of Clinical and Experimental Medicine, Federico II University Medical School, Naples, Italy.

Several lines of evidence indicate that melatonin regulates renal tubular function in addition to any postulated role for this hormone in CNS physiology. There is also recent hypothesis that melatonin plays a significant role in renal physiology, this is supported by the demonstration of specific, high-affinity melatonin receptors in the kidney of several mammalian and avian species, including humans. We hypothesized that MTNR1A genetic polymorphisms might influence the risk of kidney stones. To validate this hypothesis we resequenced the human genomic MTNR1A gene in 20 unrelated nephrolithiasis patients and in 20 healthy individuals, and performed a systematic genetic analysis. We found 5 SNPs including: two synonymous (R308R and T315T), a non synonymous change (G166E), an intronic polymorphism, and an additional SNP in the regulative region at 5 of the gene. The two synonymous changes showed a minor allele frequency of less than 5% and were removed from further statistical analysis. The remaining 3 polymorphisms with a minor allele frequency of 5% were genotyped in 150 kidney stone cases and 150 controls and used to test allele frequency differences and calculate the degree of linkage disequilibrium between these loci. The SNP -368A/G in promoter region revealed a significant difference in the genotype frequency between cases and controls (OR 0.49, 95% CI 0.26-0.92, p=0.02). The A/T SNP in intron 1, showed a strong association with kidney stone risk (alleles: OR 2.09, 95% CI 1.36-3.20, p=0.00065; genotypes: OR 5.50, 95% CI 2.00-15.09, p=0.00055). TT genotype carriers were at 5.5-fold increased kidney stone risk compared with AA genotype carriers. In conclusion, we have conducted the first mutational screen of the melatonin receptor 1A gene (MTNR1A) in relation to kidney stone formation. We have demonstrated a strong association between genetic variants in regulative region of MTNR1 gene and kidney stone formation. Of course, these results should provoke studies in other populations.

Partial *XNP* duplication causes ATRX syndrome. *D. M. Cohn[1], R. A. Pagon[2], L. Hudgins[3], C. E. Schwartz[1], R. E. Stevenson[1], M. J. Friez[1]* 1) Greenwood Genetic Center, Greenwood, SC; 2) Division of Genetics and Development, Childrens Hospital and Regional Medical Center, Seattle, WA; 3) Division of Medical Genetics, Stanford University, Stanford, CA.

Multiplex ligation-dependent probe amplification (MLPA) has become a useful methodology to identify changes in gene dosage in both research and diagnostic settings. Many commercially available MLPA kits exist for specific gene and clinical indications. Laboratories have the option of developing custom synthetic MLPA kits for applications that are not commercially available. We have taken advantage of the synthetic kit option to query cohorts of males with a suspected X-linked mental retardation (XLMR) syndrome, but are negative on sequence analysis of the associated XLMR gene. Five XLMR genes (*XNP, L1CAM, CDKL5, FGD1* and *NEMO*) were selected along with other autosomal control genes in our custom kit. Males with a specific diagnosis associated with the genes selected along with other males with mental retardation and hypotonia were analyzed for the presence of duplications. Microduplications for these XLMR genes appear to be rare as only one family was found from the screening of 1152 probands. A single microduplication of *XNP* was identified in a male proband with ATRX syndrome. Confirming the segregation, additional family members were tested and each of the affected males and the obligate carrier females were shown to have the same duplication. X chromosome comparative genomic hybridization (CGH) array studies and quantitative real time PCR (qPCR) defined the *XNP* duplication to be partial (exons 2-31) and 248 kb in length. The duplication interferes with the synthesis of full length *XNP* transcript which is apparent on Northern blot analysis and is consistent with the clinical diagnosis of ATRX syndrome. Diagnostic methodologies such as array CGH with adequate coverage in the *XNP* region should be considered in males clinically diagnosed with ATRX syndrome and normal *XNP* sequencing.

A MADA mouse model shows growth retardation, precociuous hair loss and bones anomalies. *M. Bertoli, A. Vielle, F. Lombardi, A. Botta, M. R. D'apice, A. Orlacchio, M. Federici, G. Novelli* Department of Biopathology and Diagnostic Imaging, University of Rome Tor Vergata, Via Montpellier 1, 00133 Rome, Italy.

Laminopathies are an heterogeneous group of genetic disorders, that manifest varied clinical features affecting skeletal and cardiac muscle, adipose tissue, nervous system, cutaneous tissue, and bone. Mutations in the gene encoding lamins A and C (LMNA) cause primary laminopathies, including various types of lipodystrophies, muscular dystrophies and progeroid syndromes, mandibuloacral dysplasia, dilated cardiomyopathies, and restrictive dermopathy. A rare homozygous mutation in LMNA(p.R527H) cause mandibuloacral dysplasia type A (MADA), characterized by postnatal growth retardation, dysmorphic craniofacial and skeletal features, lipodystrophy, metabolic complications and premature ageing features. The pathogenic mechanisms of this mutation in the lamins A and C are still unknown. Variable laminopathic mouse models have been constructed, nevertheless phenotypes were not always as expected.MADA transgenic mouse was generated by the random insertion of the human mutated p.R527H cDNA driven by a strong expression promoter. Transgene copy number was determined by Quantitative Real Time PCR and seemed to correlate with the severity of the phenotype. Mutant mice have slight postnatal growth retardation. Adult MADA mice have sparse and less shiny hair, with nude areas. Radiography of the transgenic mouse, compared to the wild type, showed a reduced bone density, a rounder head with shorter mandibule, and an hypoplasic clavicule. Moreover, we analyzed the behaviour of circulating MMP-9, a bone homeostasis marker altered in MADA disorder, and observed an abnormal activity and pattern in mutant mouse as seen in human patients. An animal model reproducing human phenotype is essential for an in-depth understanding of pathogenic mechanism and for the development of in vivo therapeutic assays. These preliminary results demonstrate that MADA mouse model, showing characteristic bone phenotype, will represent an interesting and useful tool for the up-coming therapeutic studies in laminopathies. This work was supported by EU grant FP6 Euro-laminopathies no. 018690.

A 300 kb terminal deletion of chromosome 4p in patient with autism indicates a possible new autism locus. *M. Velinov[1], N. Dolzhanskaya[1], H. Gu[1], G. Beldia[2], E. C. Jenkins[1], W. T. Brown[1]* 1) Dept Human Genetics, NYS Institute for Basic Research, Staten Island, NY; 2) Jervis Clinic, NYS Institute for Basic Research, Staten Island, NY.

This 16-years-old male patient was born with normal birth weight, after an uncomplicated pregnancy. The newborn period was uncomplicated. He was making consonant sounds at 9 month of age. He walked at 11 months of age and said his first words at 12 months. After his first birthday, as per his parent, his speech development slowed down and he stopped making eye contact. He had diagnosis of autism at the age of 2 that was later confirmed using the Autism diagnostic Observation Scale and the Vineland Adaptive Behavior Scale. His growth and head size were within normal range for his age. This patient was also found to have type 1 diabetes at the age of 6. He receives insulin treatments daily. This patients 10-year-old brother had juvenile diabetes as well, but his cognitive development was normal. No other family members with cognitive impairment were reported. Sub-telomeric analysis (Vysis probe) revealed a terminal 4p deletion in the proband. While enhancing the fluorescence signal of the sub-telomere hybridization, a dim signal of the sub-telomere probe was seen indicating that the breakpoint was most likely within the subtelomere probe locus. This deletion was not present in the patients brother and mother. His father was not available for testing. Further characterization of the deletion was done using 244K microarray of Agilent Inc. and additional FISH clones. The deletion was found to span the most distal 300 kb of 4p. Our patient did not have the characteristic facial features of Wolf-Hirschhorn syndrome, growth delay or microcephaly (associated with larger distal 4p deletions). A limited number of coding genes (namely ZNF595, ZNF718 and MGC26358) are located in the deleted chromosomal area. Although the contribution of these genes in brain development/functioning at this time is unclear, they may be considered as candidate contributors for autism.

Gene expression profiling of normal and pathological testis by microarray analysis. *V. Gatta[1,2], F. Raicu[3], A. P. Scioletti[1,2], A. Ferlin[4], C. Foresta[4], G. Palka[1,2], L. Stuppia[1,2]* 1) Department of Biomedical Science, G. d'Annunzio University Chieti, Italy; 2) Aging Research Center, Ce.S.I. , G. d'Annunzio University Chiet-Italy; 3) Carol Davila University of Medicine and Pharmacy Faculty of Dentistry, Bucharest,Romania; 4) Department of Histology, Microbiology and Medical Biotechnologies,University of Padova, Italy.

Male infertility is a major reproductive health problem, since approximately 15% of couples in western countries seek medical treatment for infertility. In about 10% of cases, male infertility is due to the presence of microdeletions of the Y chromosome, involving three loci on Yq (AZFa, AZFb, AZFc). Despite the large amount of data collected in the last years, the biological mechanisms leading to the disruption of the spermatogenesis in Yq deleted patients are still largely unknown. In this study we carried out a microarray analysis of the testis expression profiles of 7 patients with idiopathic infertility, 6 AZFc deleted patients and 3 control subjects (patients with obstructive azoospermia) in order to detect the specific gene networks involved in each different pathological condition. Hierarchical clustering of differentially expressed genes in testis with different forms of spermatogenesis failure as compared to testis with normal spermatogenesis evidenced the presence of more than 300 significantly downregulated and about 150 upregulated transcripts in AZFc deleted patients. Analysis of these gene clusters using IPA software revealed an interesting down-regulated gene network directly related with spermatogenesis, centred around the YBX2 gene (Y box binding protein 2), involved in RNA storage during gametogenesis. This alteration is responsible for the downregulation of the protamine1 and 2 genes evidenced in the same network analysis. This suggests that impairment of RNA storage could represent one of the biological mechanisms underlying spermatogenesis failure in patients with Yq microdeletion. In the expression profiles comparative analysis between controls and AZFc deleted patients we also observed an interesting downregulation of the CREM pathway, which is a master controller gene for spermatogenesis.

Linkage study of Ectodermal Dysplasias in Pakistani Inbred Families. *I. Ahmad, M. Rasool, M. Tariq, A. Ali, M. Bakhtiar, S. Nawaz, S. Rehman, S. Mahmood Baig* NIBGE Jhang Road P.O Box 577 Faisalabad Pakistan.

Ectodermal dysplasia (EDs) represents a large and complex group of diseases comprising more than 200 different clinical conditions. They are caused by impaired development of epidermal appendages and characterized by primary defect in at least on of the following tissue: nail, hair, teeth and sweat glands. In this study, six consanguineous families with ectodermal dysplasia were located from different areas of Pakistan having multiple affected members. Pedigrees were analyzed to determine the pattern of inheritance; in all families the disease was inherited in the autosomal recessive pattern. Short Tandem Repeat Markers (STR) were used in the exclusion mapping for the ten most common loci reported. According to results, one family with ED was linked to the EDAR gene at locus 2q13 while other families are excluded from all known loci .The families excluded to all known loci will be subjected to genome wide scan by SNP (Single Nucleotide Polymorphism) analysis using Affymetrix 250K array system to find the homozygous regions to find out the locus having the disease gene. Statistical analysis such as LOD (log of odds) will be used to validate the data obtained.

Identification of 23 TGFBR2 and 6 TGFBR1 gene mutations and genotype-phenotype investigations in 457 patients with Marfan syndrome type I and II, Loeys-Dietz syndrome and related disorders. *G. Jondeau[1,2], G. Collod-Beroud[3], L. Faivre[4], L. Gouya[2,5,6], D. Attias[2,6], B. Chevallier[2,6,7], C. Boileau[2,5,6,8], C. Stheneur[2,7]* 1) Service de cardiologie, APHP-Hôpital Bichat, Paris, France; 2) Consultation multidisciplinaire Marfan, APHP-Hôpital Bichat, Paris, France; 3) INSERM U827, Montpellier F-34000, France; 4) CHRU Dijon, Centre de genetique, Dijon,F-21000, France; 5) Laboratoire central de Biochimie, d'hormonologie et de genetique moleculaire, APHP-Hôpital A.Pare, Boulogne F-92100, France; 6) Universite Versailles-SQY, Versailles F-78000, France; 7) Service de pediatrie, APHP-Hôpital A.Pare; Boulogne F-92100, France; 8) INSERM U781, Hôpital Necker-Enfants-Malades, Paris F-75015 France.

TGFBR1 and TGFBR2 gene mutations have been associated with Marfan syndrome types 1 and 2, Loeys-Dietz syndrome and isolated familial thoracic aortic aneurysms or dissection. In order to investigate the molecular and clinical spectrum of TGFBR2 mutations we screened the gene in 457 probands suspected of being affected with Marfan syndrome or related disorders that had been referred to our laboratory for molecular diagnosis. We identified and report 23 mutations and 20 polymorphisms. Subsequently, we screened the TGFBR1 gene in the first 74 patients for whom no defect had been found, and identified 6 novel mutations and 12 polymorphisms. Mutation-carrying probands displayed at referral a large clinical spectrum ranging from the Loeys-Dietz syndrome and neonatal Marfan syndrome to isolated aortic aneurysm. Furthermore, a TGFBR1 gene mutation was found in a Shprintzen-Goldberg syndrome patient. Finally, we observed that the yield of mutation detection within the two genes was very low : 4.8% for classical MFS, 4.6% for incomplete MFS and 1% for TAAD in the TGFBR2 gene; 6.2%, 6.2% and 7% respectively in the TGFBR1 gene; in contrast to LDS, where the yield was exceptionally high (87.5%).

Fragile X premutation alleles in movement disorders. *D. Civitelli[1], E. V. De Marco[1], F. Annesi[1], P. Tarantino[1-], F. E. Rocca[1-2], G. Provenzano[1-3], V. Scornaienchi[1], V. Greco[1], G. Annesi[1]* 1) Inst Neurological Sci, National Research Council, Mangone Cosenza, Italy; 2) Institute of Neurology, University of Magna Graecia, Catanzaro, Italy; 3) Department of Neuroscience, Psychiatry and Anesthesiology, University of Messina, Policlinico Universitario, Italy.

The fragile X-associated tremor/ ataxia syndrome (FXTAS) predominantly occurs in man carrying FMR1 gene premutation alleles (55- 200 CGG rep) over age 50. Associated phenotype includes tremors, balance problems and sometimes dementia, progressively worsening over time. This picture overlaps with those of both Parkinson's disease (PD) and essential tremor (ET). We selected 203 PD patients, 30 ET patients and 370 healthy subjects. We also included two individuals (exhibiting parkinsonian symptoms) from a fragile X mental retardation pedigree, and two cases with intention tremor and postprandial hypotension. All participants had the same ethnic background and gave informed consent. The length variation within the CGG repeat was assessed by a PCR-based test. We did not found FMR1 premutation genotype in any patients with PD and ET or in any healthy controls. There were 17 distinct alleles, ranging from 19 to 37 CGG repeats. On the contrary, the two subjects with parkinsonian symptoms and family history of fragile X syndrome carried, respectively, 57 and 90 CGG repeats. Concerning the two subjects with intention tremor and postprandial hypotension, one of them had 73 CGG repeats and the second one was an uncommon mosaic for a premutation (90 CGG rep) and a normal-size allele. There is no clear biochemical link between FXTAS, PD and ET; they are thought to occur through very different disease mechanisms. However, the wide and variable FXTAS phenotype overlaps the clinical features of many neurological diseases, making diagnosis of this disorder difficult without molecular analysis. Our data show that premutated alleles are rare in PD as well as in ET. Thus the presence of postprandial hypotension or a positive family history of fragile X syndrome, beside the peculiar T2-hyperintense signal in middle cerebellar peduncles, can be considered an important indication for FMR1 expansion genetic testing.

A computational evolution system for the genetic analysis of epistasis. *J. Moore, P. Andrews, C. Greene*
Computational Genetics Laboratory, Department of Genetics, Dartmouth College, Lebanon, NH.

Common human diseases are complex and likely the result of nonlinear interactions between multiple different DNA sequence variations. One goal of human genetics is to use data mining and machine learning methods to identify sets of discrete genetic attributes that are predictive of discrete measures of health in human population data. A variety of different computational intelligence methods based on artificial evolution have been developed and applied in this domain. While artificial evolution approaches such as genetic programming show promise, they are only loosely based on real biological and evolutionary processes. It has recently been suggested that a new paradigm is needed where artificial evolution is transformed to computational evolution by incorporating more biological and evolutionary complexity into existing algorithms. Computational evolution systems have been proposed as more likely to solve problems of interest to biologists and biomedical researchers. To test this hypothesis, we developed a prototype computational evolution system for the analysis of human genetics data capable of evolving operators of arbitrary complexity. Preliminary results suggest that more complex operators result in better solutions. Here we introduce modifications including a simpler stack-based solution representation, the ability to maintain and use an archive of solution building blocks, and a simpler set of solution operator building blocks capable of learning to use pre-processed expert knowledge. A parameter sweep suggests that operators that can use expert knowledge or archival information outperform those that cannot. This study supports the idea that complexity matters and thus the consideration of computational evolution for bioinformatics problem-solving in the domain of human genetics.

Findings from a UK National and International Service for Germline TP53 mutational analysis that have implications for genetic testing and counselling. *N. Sodha[1], S. Furnell[1], R. Eeles[2,1]* 1) Cancer Genetics, Royal Marsden NHS Hospital Foundation Trust, Sutton, Surrey, United Kingdom; 2) Section of Cancer Genetics, Institute of Cancer Research, Sutton, Surrey, SM2 5NG, United Kingdom.

Around 75% of families with classical Li-Fraumeni syndrome (LFS) have a germline mutation in TP53 identifiable by direct sequencing of the coding region and splice sites. We have used Multiplex Ligation dependent Probe Amplification (MLPA) to screen affected individuals from 24 classical LFS families, 62 Li-Fraumeni Like (LFL) families (Birch Definition), 13 affected cases from families with very early onset breast cancer at<30yr with or without some family history (FH) and 31 cases with multiple tumours with or without some FH, that were negative for a germline TP53 mutation identifiable by sequencing. 6 cases from classical LFS families 25%) and one case with multiple tumors with some FH of cancer were found to have a large deletion. These findings demonstrate that a substantial fraction of LFS families have a large deletion in TP53 indicating that this type of analysis should be included for a comprehensive analysis of the gene in LFS families. a low penetrance TP53 mutation, R337H, initially reported to be associated with cases with adrenocortical cancer without a family history of cancer in the brazilian population and subsequently in cases with a LFL family structure in the same population, was identified in individuals from four different UK families in out laboratory. Two of the families have cases with adrenocortical tumors and there are individuals with other early onset cncer in all four of these families. These findings have implications for genetic counselling.

Distinct effects of an HLA-C associated SNP on HIV pathogenesis. *R. Thomas[1], Y. Qi[1], D. Ge[2], J. Fellay[2], J. Phair[3], J. Goedert[4], S. Buchbinder[5], G. Kirk[6], A. Telenti[7], B. Walker[8], S. Deeks[9], D. Goldstein[2], M. Carrington[1]* 1) Cancer and Inflammation Program, SAIC-Frederick, NCI-Frederick, Frederick, MD; 2) Center for Population Genomics & Pharmacogenetics, Duke University, Durham, NC; 3) Department of Medicine, Northwestern University Medical School, Chicago, IL; 4) Viral Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Rockville, MD; 5) San Francisco Department of Public Health, HIV Research Section, San Francisco, CA; 6) Department of Epidemiology, Johns Hopkins University, Bloomberg School of Public Health, Baltimore, MD; 7) Institute of Microbiology, University of Lausanne, Lausanne, Switzerland; 8) Partners AIDS Research Center and Infectious Disease Division, Harvard Medical School, Boston, MA; 9) San Francisco General Hospital, San Francisco, CA.

Killer immunoglobulin-like receptors (KIR) are receptors on Natural Killer (NK) cells and bind to specific HLA class I ligands. Interactions between KIR and their HLA ligands have been implicated in NK cell mediated control of HIV-1 replication. A variant located 35kb upstream of the HLA-C gene(-35C/T) was previously shown to associate with high HLA-C mRNA expression and low viraemia. Analysis of this SNP in over 1500 HIV^+ individuals of Caucasian ancestry revealed a significant effect of the -35CC vs TT on mean viral load and a weaker protective effect with progression to late AIDS outcomes. HLA-C alleles are ligands for certain KIRs, including the activating KIR2DS receptors, which are present in some, but not all individuals. We observed that in the absence of KIR2DS, high HLA-C expression (as defined by -35C/T SNP) associated with slow progression to late outcomes of HIV infection, including death (CC/CT no KIR2DS was protective compared to TT no KIR2DS). However, in presence of KIR2DS, HLA-C levels had no effect on progression. Also, KIR2DS did not modulate the effect of -35C/T SNP on viral load. Thus, activating KIR does not influence the protective effect of -35C (high HLA-C levels) on early control of HIV-1, but has a significant effect on AIDS progression in late chronic disease. Funded by NCI Contract N01-C0-12400.

Genotype Frequencies in the MPS I Registry. *G. Cox[1], J. E. Wraith[2], C. Whitley[3], F. Wijburg[4], N. Guffon[5]* 1) Genzyme Corp, Cambridge, MA; 2) Royal Manchester Children's Hospital, Manchester, UK; 3) University of Minnesota, Minneapolis, MN; 4) Academic Medical Center, Amsterdam, The Netherlands; 5) Hôpital Edouard-Herriot, Lyon, France.

Background: The MPS I Registry includes over 700 patients and offers a unique resource to study mutation frequencies and genotype-phenotype correlations. MPS I patients are historically grouped into three phenotypes based on subjective assessment of disease severity: Hurler (severe), Hurler-Scheie (intermediate), and Scheie (attenuated). Although several mutations correlate with disease severity, most patients have at least one private mutation. **Methods:** Reported patient mutations were revised according to standardized nomenclature. Mutations were summarized by frequency, and genotype frequencies were assessed by phenotype. **Results:** IDUA gene mutations were reported for 46% of Hurler, 44% of Hurler-Scheie, and 41% of Scheie patients. Of 107 unique mutations reported in 293 patients, the 10 most common were: W402X (39%), Q70X (13%), P533R (7%), L490P (5%), A327P (3%), L218P and Q380R (2%); and T388R, S633L, and R628X (1%). Of patients with 2 reported mutations (87% of total), 64% were Hurler, 26% Hurler-Scheie, 8% Scheie, and 2% undetermined. Three genotypes were present in 44% of 184 Hurler patients: W402X/W402X (25%), W402X/Q70X (15%), and Q70X/Q70X (4%). Of 71 Hurler-Scheie patients, the 2 most common genotypes were L490P/L490P (18%) and P533R/P533R (7%). Scheie patients had no common genotypes. Patients with 2 nonsense mutations almost invariably had Hurler syndrome. One Hurler-Scheie and 2 Scheie patients had a mild R89Q mutation. Three Hurler, 5 Hurler-Scheie, and 1 undetermined patient were homozygous for the variable P533R mutation, which was combined with W402X in 3 Hurler, 3 Hurler-Scheie, and 1 Scheie patient. **Conclusions:** Mutation frequencies and genotypes reported in the MPS I Registry reflect those in the literature. The probability of a mutation being severe or mild may be estimated by pair-wise matching of mutations with known phenotypes. Genotyping may help in treatment decisions for affected infants and presymptomatic newborns identified by newborn screening.

Mothers genome or maternally-inherited genes acting in the fetus influence gestational age in familial preterm birth. *J. Plunkett, M. Feitosa, M. Trusgnich, T. Rice, I. Borecki, L. Muglia* Washington Univ, St Louis, MO.

Infants born before term (<37 weeks) have an increased risk of neonatal mortality as well as other health problems. The increasing rate of preterm birth in recent decades, despite improvements in health care, creates an impetus to better understand and prevent this disorder. While multiple lines of evidence suggest the importance of genetic contributors to risk of preterm birth, the nature of the genetic component has not been identified. In this study, we perform segregation analyses to identify the best fitting genetic model for gestational age, a quantitative proxy for preterm birth. Because either mother or infant can be considered the proband from a preterm delivery and there is evidence to suggest that genetic factors in either one or both may influence the trait, we performed segregation analysis for gestational age either attributed to the infant (infants gestational age, n=1130), or the mother (by averaging the gestational ages at which her children were delivered, n=191), using 96 multiplex preterm families. Using the Pedigree Analysis Program, likelihood ratio tests identified the parent of origin and mixed free (non-Mendelian) transmission models as the most parsimonious models for infant and mother analysis, respectively. These data lend further support to a genetic component contributing to birth timing since sporadic (ie. no familial resemblance) and nontransmission models, in which gestational age is attributed to environmental factors alone, are strongly rejected ($p<0.001$). Heritability estimates corroborate the general importance of genetics in birth timing (infant 0.33 (95% CI: 0.24, 0.42); mother 0.44 (95% CI: 0.11, 0.77)). Infant-based analyses support a model in which mothers genome and/or maternally-inherited genes acting in the fetus are largely responsible for birth timing, with a smaller contribution from the paternally-inherited alleles in the fetal genome. In stratified analyses, evidence for heterogeneity among black and white races was observed for both traits ($p<0.001$). Overall, our findings suggest that genetic influences on birth timing are important and likely complex.

Glycosylation Defects in Muscular Dystrophy. *S. Sparks[1,2], A. Kesari[2], E. Hoffman[2]* 1) Division of Genetics & Metabolism, CNMC, Washington, DC; 2) Research Center for Genetic Medicine, CNMC, Washington, DC.

Abnormal glycosylation of -dystroglycan (-DG) underlies the pathology of dystroglycanopathies. The clinical phenotype ranges from congenital onset of muscular dystrophy (CMD) with CNS and eye involvement, to a later onset form of limb girdle muscular dystrophy (LGMD). To date, 6 genes have been identified which alter the glycosylation pattern of -DG, all of which are known or putative glycosyltransferases. However, with the anticipated 10-15 steps in the glycosylation of -DG, there are more to be identified.

Methods: The muscle biopsy database of Dr. Eric Hoffman contains over 5000 samples that were referred for diagnostic testing of muscular dystrophy. Standard testing for dystrophin, merosin, dysferlin, and -sarcoglycan were completed. This database was screened for patients without a diagnosis for those potentially with a dystroglycanopathy. Identification of samples with a dystroglycanopathy was done using a combination of immunohistochemisty and sequencing.

Results: Screening the muscle biopsy database yielded 113 samples with unknown muscular dystrophy and clinical features and muscle histology consistent with CMD. These samples were screened for a glycosylation defect using immunohistochemistry, followed by molecular and biochemical characterization. A separate molecular screen for *FKRP* mutations was performed on 1088 patients with unknown LGMD. The c.826C>A (p.L276I) mutation was found in 26 patients, of which 10 were homozygous. A second mutation was identified in an additional 6 patients. In those with two mutations, glycosylation status of -DG has been analyzed.

Conclusion: Screening patients with unknown CMD using immunohistochemistry for abnormally glycosylated -DG on muscle tissue yields a positive result in 25% of patients. In contrast, patients with confirmed molecular defects in *FKRP* may have nearly normal glycosylation of -DG by immunohistochemistry. Therefore, screening LGMD patients for mutations in *FKRP* is a reasonable first step to evaluate LGMD patients for a dystroglycanopathy.

Miglustat in Niemann-Pick disease Type C (NPC): long-term data from a clinical trial. *M. C. Patterson[1], D. Vecchio[2], E. Jacklin[3], J. E. Wraith[3]* 1) Mayo Clinic, Rochester, USA; 2) Columbia University, New York, USA; 3) Royal Manchester Childrens Hospital, UK.

Previous clinical trial findings indicated that miglustat slows disease progression in NPC patients up to 24 months of treatment.[1,2] Here we report the long-term data in adult and juvenile patients who received up to 48 months of treatment with miglustat. Patients aged 12 years who completed an initial 12-month, open-label, randomized phase (either on miglustat 200 mg t.i.d. or standard care) and a further 12-month open-label extension period were offered continued miglustat treatment. Of 19 patients fulfilling these criteria, 16 (84%) continued with further treatment (mean SD [range] age: 22.6 9.4 [12-42] years; 44% female). The number of patients completing the Month 36, 42 and 48 visits were 15 (94%), 11 (69%) and 9 (56%), respectively. Median (range) treatment duration was 48.2 (27.1-67.5) months. Swallowing capacity (among 14 evaluable patients) was improved or stable in 11 (79%) patients (with water), 12 (86%) patients (with puree), and 13 (93%) patients (with both soft lumps and cookies) at last assessment. Ambulation Index (among 12 evaluable patients) remained stable in 8 (67%) patients at last assessment (mean change [95%CI] +0.6 [-0.2, +1.4]). The most frequent adverse events were diarrhea and weight decrease, each observed in 50% of patients, in contrast to 89% experiencing diarrhea and 68% with weight decrease during the first 24 months. Mean SD (range) weight change from baseline to last value was +0.56 8.10 kg (-9.3 to +21.0 kg). Three patients discontinued; none because of adverse events. Four patients did not complete the end of study visit. These data indicate a favourable clinical response with miglustat in adult and juvenile NPC patients, with stabilization of key parameters of disease progression seen over a median exposure of 48 months. Safety and tolerability with miglustat 200 mg t.i.d. in NPC were consistent with previous findings with miglustat 100 mg t.i.d. in type 1 Gaucher disease. [1]Patterson et al. Lancet Neurol 2007;6:765-72. [2]Patterson et al. Presented at the 57th annual conference of the ASHG, 2007, abstract 2253.

Harnessing the power of human perception for exploration of analytical results from genome-wide association studies. *R. Cowper, J. Moore* Computational Genetics Laboratory, Department of Genetics, Dartmouth College, Lebanon, NH.

Interpretation of data analysis results from genome-wide associations studies (GWAS) is a daunting challenge. In most cases, the geneticist or epidemiologist relies only on his or her minds eye to envision the vastness and complexity of the human system. This process of analysis and exploration can be greatly enhanced if the power of visualization technologies and information design are used to harness the innate pattern recognition capabilities of human perception. Increasing the synergy between the geneticist and the computer will doubtlessly aid in making novel discoveries. However, information visualization faces several challenges in the field of human genetics due to the large volume, high dimensionality and interconnectedness of the data. In the present study we address some of these challenges and present a conceptual and software framework with which to tackle further challenges. We hypothesize that by representing each SNP in a GWAS as a tree or some other object in a natural landscape we will improve our ability to quickly identify interesting results by relying on our innate pattern recognition capabilities. To test this hypothesis we developed a system for visualizing statistical results as a natural landscape using Java-3D. Here, we represented each SNP as a tree with features like height, shape and color dependent on values of different statistical results such as a chi-square test of independence and ReliefF. We show that visually interesting patterns emerge when trees are drawn using the result of multiple different statistical measures of association. We further show how information from chromosomal location, Gene Ontology and biochemical pathway can be incorporated into these landscapes thus providing a biological context to the visualization. This study represents the first step towards a platform that harnesses the power of human perception for interpreting statistical results from GWAS.

Identification of a mutation in the *UFM1-specific peptidase 2* gene, *UFSP2*, that is linked to Beukes Hip Dysplasia. *C. M. Watson[1], P. Beighton[2], R. Ramesar[2], J. B. J. van Meurs[3], R. Donn[1], G. Wallis[1]* 1) The School of Translational Medicine, The University of Manchester, Oxford Road, Manchester, M13 9PT, United Kingdom; 2) Division of Human Genetics, University of Cape Town, Faculty of Health Sciences, South Africa; 3) Department of Internal Medicine, Erasmus MC, Rotterdam, The Netherlands.

Beukes hip dysplasia (BHD) is a rare, autosomal dominant disorder that leads to severe premature osteoarthritis of the hip that was originally identified in an extended South African family of Dutch origin. Previous studies have linked the disorder to a 2.79 Mb region on chromosome 4q35.

Linkage analysis refined the linked allele to a 1.33 Mb region encompassing 18 genes. Sequence analysis of the exons of these genes identified a heterozygous T to C transition within exon 8 of the *UFM1-specific peptidase 2* (*UFSP2*) gene that predicted a Y290H amino acid substitution in the encoded protein. Segregation of the C allele with the BHD phenotype gave a LOD score of 10.4. Sequence alignments demonstrated that this tyrosine was highly conserved across multicellular organisms. Further, the T/C sequence change was not identified following screening of a control population of 360 Dutch individuals.

UFSP2 has been demonstrated previously to cleave the C-terminal end of a member of the family of ubiquitin-like molecules, ubiquitin-fold modifier 1 (UFM1). We found that introduction of the histidine mutation abolishes UFSP2 cleavage of UFM1 *in vitro*. Preliminary studies in the chondrogenic ATDC5 cell line suggest a correlation of UFSP2 expression and chondrogenesis.

Genetic and preliminary functional studies indicate a role for the UFSP2 Y290H mutation in the aetiology of BHD. Additional studies are underway to investigate this possibility further.

Microcephalia Vera : Neuropsychologic and neuroradiologic findings of 9 children with ASPM gene mutations.

S. Passemard[1,2], M. Schaer[3], L. Titomanlio[1], K. Hernandez[1], T. Billette de Villemeur[4], S. Odent[5], B. Isidor[6], O. Boesflug Tanguy[7], A. Megarbane[8], JL. Alessandri[9], M. Abramowicz[10], B. Gerard[1], S. Eliez[3], M. Elmaleh[11], A. Verloes[1,2] 1) Genetic Department, Robert Debré Hospital, Paris, France; 2) INSERM U676, Robert Debré Hospital, Paris, France; 3) Department of Psychiatry, University of Geneva School of Medecine, Geneva, Switzerland; 4) Child Neurology Department, Trousseau Hospital, Paris, France; 5) Genetic Department, Rennes Hospital, France; 6) Genetic Department, Nantes Hospital, France; 7) Genetic Department, Clermont Ferrand Hospital, France; 8) Saint Joseph University, Beyrouth, Liban; 9) Pediatric Department, Saint Denis de la Réunion Hospital, France; 10) Genetic Department, Université Libre, Bruxelles, Belgien; 11) Radiology Department, Robert Debré Hospital, Paris, France.

Primary Human Microcephaly (MCPH previously refered to as Microcephalia Vera) is the autosomal recessive form of primary microcephaly. Affected individuals have a reduced brain size. Cerebral cortex is most severly affected by this reduction. Despite this marked reduction in size, the gyral pattern is relatively well preserved, with no major abnormality in the cortical cytoarchitecture. Individuals have mild to severe mental retardation. Six autosomal recessive loci and four genes have been identified (MCPH1, 3, 5 or ASPM, and 6). We report on the neurological and radiological phenotype of 9 European MCPH patients with ASPM gene mutations. Their intellectual quotien is between 35 and 70. Of all patients, 7 have gyral simplification and one child has a large unilateral polymicrogyria, the latter has not been described in this disease entity. Mental retardation is more severe when the head circumference is smaller, when the gyral pattern is simplified, and when a disorder of neuronal migration is apparent. Despite their mental retardation, the memory capacities of affected children is remarkably preserved. The results of our study suggest a phenotypic continuum between MCPH and microcephaly with simplified gyral pattern. There is a correlation between mental retardation severity and head circunference and gyral pattern simplification.

Regulatory SNPs in double strand break repair genes and their impact in childhood leukemia susceptibility. *M. Bourgey, N. N'Diaye, V. Weth, J. Healy, K. Benhamza, D. Sinnett* Hemotologie-Oncologie, Centre de recherche CHU Sainte-Justine, Universite de Montreal, Montreal, Quebec, Canada.

Acute lymphoblastic leukemia (ALL), the commonest form of childhood cancer, accounts for approximately 25% of all pediatric tumors. Considering that genetic instability is a hallmark of leukemia, that interindividual variation in DNA repair capacity is observed in the general population and that variation in DNA repair capacity is probably genetically determined, we propose that sequence variations in DNA repair genes might constitute a cancer risk factor. In this report, we studied the impact of regulatory SNPs (rSNPs) in genes encoding components of the double strand break (DSB) repair system hypothesizing that variation in regulatory sequences of such finely regulated genes will indeed play significant roles in cancer risk. In a case control study we genotyped 12 rSNPs in 6 genes for a sample of 330 cases and 320 controls all of French-Canadian origin. For each gene, the combination test was used to select the most significantly associated subset of rSNPs. Their haplotypic and genotypic effects on the childhood ALL were then modeled using the Marker Association Segregation Chi-square (MASC) method. Using a logistic regression analysis we also tested for the presence of interactions between selected subsets. We observed an associated combination of rSNPs in two genes, BRCA2 and KU80 ($p = 0.005$ and $p = 0.024$ respectively). The modeling of BRCA2 rSNPs suggested the role of three genotypic groups (GRR = 1 ; 1.52 [1.10 ; 2.04]; 2.27 [1.52 ; 3.28]). For KU80, we showed a dose effect of the rSNP -297T allele (GRR = 1; 1.31 [1.21; 1.86]; 2.02 [1.75 ; 2.88]). There was no evidence of a significant interaction between the two genes ($p = 0,73$). The lack of interaction between these two genes and the fact they belong to two distinct repair mechanisms suggest that quantitative changes in at least one DSB repair component is required to modify individuals risk of childhood ALL. We are now performing functional validation assays of the rSNPs and the related promoter constructs in order to gain knowledge about the biological significance of the observed associations.

Fetal Ventriculomegaly secondary to large choroid plexus cysts: Prenatal findings and postnatal outcome. *T. Friedberg[1,2], K. Chong[1,2,4], A. Toi[3], K. Fong[3], D. Chitayat[1,2,4]* 1) Prenatal Diagnosis and Medical Genetics, Mount Sinai Hospital, Toronto, ON, Canada; 2) Obstetrics and Gynecology, Mount Sinai Hospital, Toronto, ON, Canada; 3) Department of Diagnostic Imaging, Mount Sinai Hospital, Toronto, ON, Canada; 4) Clinical and Metabolic Genetics, The Hosptial for Sick Children, Toronto, ON, Canada.

Choroid plexus cysts (CPCs) are sonographically detectable folds of the epithelial lining of the choroid plexus. In most cases, fetal CPCs are normal variants with a natural history of regression during the third trimester of pregnancy or after delivery. Outcome studies of isolated CPCs have reported no associated developmental delay or other related problems. However, when detected prenatally, CPCs have been associated with an increased risk for fetal trisomy 18. Enlargement of the fetal cerebral lateral ventricles caused by large CPCs, to the best of our knowledge, has not been reported. We report on six cases detected prenatally with a large isolated CPCs that resulted in ventriculomegaly (lateral ventricular width greater than 10 mm). All cases had normal first or second trimester screening for trisomy 18 and all detailed fetal ultrasounds subsequently showed multiple CPCs measuring 10 mm or more. Four patients had amniocentesis with normal karyotypes and the other two patients declined invasive testing. All cases had negative screen for intrauterine infection. Serial ultrasounds were performed during the pregnancy for surveillance of the ventriculomegaly. All cases resulted in healthy newborns delivered at term. Two of the cases have had postnatal follow-up to almost 2 years of age and have not developed any medical or neurological problems. To the best of our knowledge, this is the first report of ventriculomegaly caused by large CPCs. It shows that ventriculomegaly secondary to large CPCs is a different category of mild ventriculomegaly and is associated with normal outcome. This information is important for counselling couples with similar prenatal findings.

FAM-MDR: A flexible method of multifactor dimensionality reduction for high-order genetic interaction detection in related individuals. *K. Van Steen[1,2], M. Calle[3], V. Urrea[3], N. Malats[4]* 1) Department of Electrical Engineering and Computer Science, University of Liège, Liège, Belgium; 2) Department of Human Genetics, University of Leuven, Leuven, Belgium; 3) Department of Systems Biology, Universtiat de Vic, Vic, Spain; 4) Centro Nacional de Investigaciones Oncológicas, Madrid, Spain.

In the search for high-order genetic interactions parametric approaches have severe limitations when there are too many independent variables in relation to the number of observed outcome events. The nonparametric Multifactor Dimensionality Reduction method, MDR (Ritchie et al. 2001), has achieved a great popularity in genetic association screening. It tackles the dimensionality problem of interaction detection by reducing the dimension to one, after pooling multi-locus genotypes into two groups of risk: high and low. However, when analyzing gene interactions in case-control studies more flexible approaches which allow adjustment for confounding variables and for main effects would be preferred. This is the case of the Model-Based Multifactor Dimensionality Reduction Method, MB-MDR (Calle et al. 2007), which is a (potentially) model-based version of MDR that can deal with continuous measurements. Martin et al. (2006) combined the MDR method with the genotype-Pedigree Disequilibrium Test (MDR-PDT) to allow identification of single-locus effects or joint effects of multiple loci in nuclear families. We propose a novel multifactor dimensionality reduction strategy for genetic interaction association analysis in families of any structure. First the individual environmental residuals are estimated using an additive polygenic model, similar to the first step in the GRAMMAR approach of Aulchenko et al. (2007). Second, MB-MDR is applied to these residuals. The approach is evaluated and validated using simulated and real-life data.

Main Reference: Calle ML, Urrea V, Malats N, Van Steen K. (2007) MB-MDR: Model-Based Multifactor Dimensionality Reduction for detecting interactions in high-dimensional genomic data. Technical Report n.24. Department of Systems Biology. Universitat de Vic.

The pharmacogenetics of Nevirapine hepatotoxicity: a retrospective study in a Mozambique population. *C. Ciccacci[1], P. Borgiani[1], S. Ceffa[2], E. Sirianni[1], M. C. Marazzi[3], G. Novelli[1], L. Palombi[4]* 1) 1Department of Biopathology and Diagnostic Imaging, Section of Genetics, School of Medicine, Tor Vergata University, Rome, Italy; 2) DREAM Program, Rome, Italy; 3) LUMSA University, Rome, Italy; 4) Department of Public Health, Tor Vergata University, Rome, Italy.

NNRTIs (nonnucleoside reverse transcriptase inhibitors) nevirapine and efavirenz are widely used to treat HIV-1 infection, but adverse reactions are common (2-10 per cent), in particular the risk of nevirapine associated hepatotoxicity. Previous studies have reported associations between nevirapine-induced hepatotoxicity and some genetic variants of these genes, even if results are not always concordant. We have conducted a pharmacogenetic retrospective study on a cohort of 161 pregnant women initiating antiretroviral treatment in 3 DREAM (Drug Resource Enhancement against AIDS and Malnutrition) clinical centers in Mozambique. In particular we have compared 73 women who presented nevirapine-induced hepatotoxicity (cases) and 88 with no hepatotoxicity (controls). We considered the following 6 polymorphisms (SNPs): MDR1 T3421A, MDR1 C3435T, CYP2B6 G516T, CYP2B6 T983C, CYP3A4 G392A, CYP3A5 A6986G. We also performed haplotypes analysis for the SNPs in the same gene. The MDR1 C3435T SNP presents a significant association with epatotoxicity (p=0.03) with the variant T allele showing a protective effect (O.R. 2.27). The other SNPs are not associated or present a border line P value. The association does not increase condidering haplotypes rather than single SNPs. Analyzing by ANOVA the differences in AST and ALT maximum value among the different genotypes of each locus, it is evident an interesting dose-allele dependent trend for most of the studied SNPs, in particular for CYP2B6 G516T, even if these differences are not statistically significant maybe due to the small sample size. These preliminary results confirm the important contribution of MDR1 C3435T SNP to predict nevirapine-induced hepatotoxicity risk and, in the same time, suggest the necessity of further studies to identify new genes/variants involved in nevirapine adverse reaction.

Ethnicity-Confirmed Genetic Structure in New Hampshire. *C. Sloan, A. Andrew, E. Duell, M. Karagas, J. Moore*
Computational Genetics Laboratory, Department of Genetics, Dartmouth College, Lebanon, NH.

Genetic population structure is known to result from shared ancestry. Though there have been several studies of genetic structure within and among different geographic regions and ethnic groups, little is known of the genetic structure of highly admixed US populations or whether the structure is concordant with self-reported ancestry. In this study, 1529 single nucleotide polymorphisms (SNPs) from 864 healthy control individuals from New Hampshire were measured as part of a bladder cancer epidemiology study. The SNPs were from approximately 500 cancer susceptibility genes scattered throughout the genome. Of these, 960 Tag SNPs were used to cluster individuals using the Structure algorithm for between 2 and 5 subpopulations. Subtle genetic structure was found, suggesting the appropriate number of subpopulations to be either 4 or 5 (FSTs 4 populations: 0.0377, 0.0399, 0.0363, 0.0340; 5 populations: 0.0452, 0.0536, 0.0585, 0.0534, 0.0521). We coded the individuals self-reported ancestries in a genotype fashion (i.e. 0= not reporting that ancestry, 1= reporting part that ancestry, 2= reporting only that ancestry) and conducted a Spearmans rank correlation between each ancestry and the structure q value, which represents the proportion of an individual that originated from a certain genetic subpopulation. Those of Russian, Polish and Lithuanian ancestry most consistently clustered together. The ancestry results support either 4 or 5 subpopulations. In order to investigate linkage disequilibrium (LD), the complete set of SNPs from the 7 most densely genotyped genes were used to make haploview plots between the different groups. The results vary by gene, though for one gene in particular, GHR, the results are very different for 4 subpopulations. These results suggest that despite New Hampshires admixture and presumed homogeneity, there are 4 or 5 distinct genetic subgroups within the population that can be linked to self-reported ancestry and display differences in patterns of LD.

Association of Paternal Age and Risk for Congenital Anomalies from the National Birth Defect Prevention Study, 1997-2004. *R.Fisk Green[1], K. S. Crider[1], R. S. Olney[1], O. Devine[1], N. Archer[2], A. Olshan[3], S. K. Shapira[1], National Birth Defect Prevention Study* 1) National Center on Birth Defects and Developmental Disabilities; CDC; Atlanta, GA; 2) Texas Dept. of State Health Services; Austin, TX; 3) Dept. of Epidemiology; University of North Carolina; Chapel Hill, NC.

Previous studies have found associations between advanced paternal age and increased risk for dominant single gene disorders in offspring. The relationship between paternal age and the risk of birth defects not caused by recognized single gene disorders or chromosomal abnormalities remains unclear. We used 1997-2004 data from the National Birth Defects Prevention Study (NBDPS), a case-control study, to look for associations between paternal age and birth defects of unknown etiologies for birth defect categories with 100 case children. We performed logistic regression analyses, including paternal age, paternal age^2, maternal age, and maternal age^2 as continuous variables, while controlling for gravidity, periconceptional folic acid use, maternal body mass index, paternal birthplace, paternal education, paternal race and ethnicity, singleton/multiple birth, maternal smoking, maternal alcohol use, paternal drug use, use of assisted reproductive technology, and previous stillbirth or miscarriage. Preliminary analyses showed associations with paternal age for encephalocele (odds ratio (OR) = 1.26 per year increase in paternal age, 95% confidence interval (CI) 1.03-1.53), cataracts (OR = 1.19 per year, 95% CI 1.02-1.39), esophageal atresia (OR 1.11 per year, 95% CI 1.00-1.23), cleft palate (OR = 1.02 per year, 95% CI 1.00-1.04), and diaphragmatic hernia (OR = 1.04 per year, 95% CI 1.01-1.06). We assessed the need to include the interaction of maternal and paternal age in the models and an interaction term was required in models of the association of paternal age with gastroschisis, omphalocele, and spina bifida. In general, younger paternal age showed an association with gastroschisis, while advanced paternal age was associated with omphalocele and spina bifida. Our findings suggest that paternal age may be a risk factor for some birth defects with complex etiologies.

Chromosomal anomalies and Dandy-Walker syndrome. *M. Martin[1], A. Wagner[2], E. Knudtson[2], H. Zhang[1], J. Lee[1], J. Mulvihill[1], S. Li[1]* 1) Dept. of Pediatrics. University of Oklahoma Health Sciences Center, Oklahoma City, OK; 2) Dept. of Ob./Gyn. University of Oklahoma Health Sciences Center, Oklahoma City, OK.

Although there is variation in the diagnosis of Dandy-Walker syndrome, it is clinically defined by a triad of malformations: (1) agenesis of the cerebellar vermis (partial or complete), (2) an enlarged posterior fossa with displacement of the tentorium and the torcular and lateral sinus, and (3) cystic dilation of the fourth ventricle. The patients with Dandy-Walker syndrome have a wide range of clinical features; from severe mental retardation and physical handicaps to normal behavior and function. The etiology of Dandy-Walker syndrome in the majority of cases is unknown. There are sporadic case reports of Dandy-Walker syndrome associated with chromosomal anomalies, including whole chromosomal trisomies, partial trisomies/duplications, and deletion of various chromosomal regions of various sizes. In this report, we have searched PubMed and have identified all the reported Dandy-Walker cases up to this date with chromosomal anomalies. We have also made a map of all the identified chromosomal anomalies; this information provides valuable data for our future array CGH analysis in those patients with Dandy-Walker syndrome.

DNA repair genotypes and associated phenotypes in breast cancer. *L. J. Ricks-Santi[1,2], Y. Yang[2], F. Seillier-Moiseiwitsch[2], J. Freudenheim[3], C. Isaacs[2], M. Schwartz[2], R. Dumitrescu[2], C. Marian[2], J. Nie[3], D. Vito[3], M. Trevisan[3], S. Edge[3], P. Shields[2]* 1) National Human Genome Center, Howard University Cancer Center, Washington, DC; 2) Lombardi Comprehensive Cancer Center, Georgetown University Medical Cancer, Washington, DC 20016; 3) Department of Social and Preventive Medicine, University at Buffalo, Buffalo, NY 14214.

This study investigated women from high-risk breast cancer families and a sporadic breast cancer case-control study for associations with specific BRCA1 SNPs and haplotypes, Rad51 SNPs, and deficient DNA repair. The mutagen sensitivity assay, used to measure heritable DNA repair capacity, was applied to EBV-immortalized cell lines from 138 women from high-risk breast cancer families with known BRCA1 mutations. The assay was studied in relation to associations with genotypes and haplotypes for BRCA1 and Rad51. Positive associations were then tested as predictors of breast cancer risk in a population-based case-control study (n= 1165 cases and 2170 controls). It was found that high-risk women with breast cancer had more mean breaks per cell than those without breast cancer. The Rad51 5UTR 135 variant genotype was also associated with high MBPC. In this sample, there also was an increased risk for MBPC with the BRCA1 D693N variant genotype, but this was not statistically significant. There was no association with MBPC for the BRCA1 Q356R and E1038G genotypes or BRCA1 haplotypes. In this sample set, the Rad51 5UTR 135C variant allele and D693N allele were also examined in a population-based case-control study of breast cancer, but no association was found even after stratifying by menopausal status and adjusting for confounding variables. The results indicate that the Rad51 5UTR 135C allele and D693N allele may be modifying genotypes for the penetrance of BRCA1 mutation carriers by affecting DNA repair efficiency. This study provides data to justify a well-designed epidemiology study of the MSA as a predictive phenotype in women from high-risk breast cancer families and breast cancer risk where genetic modifiers could have implications for the clinical management of BRCA1 mutation carriers.

Theory and Methods for Association Testing using Case Trios and Unrelated Controls. *M. Lee[1], Y. Lin[1,2], E. Feingold[1,3]* 1) Department of Biostatistics, University of Pittsburgh, Pittsburgh, PA; 2) School of Medicine, University of Pittsburgh, Pittsburgh, PA; 3) Department of Human Genetics, University of Pittsburgh, Pittsburgh, PA.

The case-control study design and the transmission disequilibrium test (TDT) design are commonly used in genetic association studies. When population stratification exists, simple case-control statistics are biased, but the TDT has robust type I error. Many studies, however, have both case trios and unrelated controls (and/or unrelated cases) available, and it is not always clear how to optimally analyze this combination design. Epstein et al.(2005) proposed a likelihood-based method for combining case-control and TDT data. They demonstrated that their method is more powerful than a TDT test alone, but did not make a comparison to a case-control test, We discuss the theory of the combination design and the likelihood test, and then use simulation studies to examine how much power is gained by the likelihood approach under different genetic models and stratification scenarios.

Epistasis in Digital Organisms. *A. Tyler, J. Moore* Computational Genetics Laboratory, Department of Genetics, Dartmouth College, Lebanon, NH.

Many of the genes that contribute to complex diseases do so not through independent effects, but through epistatic interactions with other genes. Increasing numbers of studies are acknowledging the importance of epistasis in human genetic architecture and are beginning to discover novel gene-gene interactions associated with complex diseases. It is thought that there may be tens, hundreds, or even thousands of genes interacting to contribute to risk of complex disease. Finding these interactions among the thousands of genes in a genome-wide dataset is a non-trivial task. There are several major barriers that need to be overcome before the complexity of epistasis can be effectively addressed in practice. One of these barriers is that there is a lack of general understanding as to how epistatic interactions fit into the larger picture of genetic architecture. We know that epistatic interactions are a system-wide feature of biological genomes, but what are the patterns in which these interactions arise? How much do they contribute to complex phenotypes? Here we address these questions through the lens of complex systems and digital biology. Digital organisms are computer programs that evolve over time through a process of natural selection that includes mutation, replication, and selection of the most fit organisms. Over many generations digital organisms evolve a complex genetic architecture that in many aspects resembles the genetic architecture of biological genomes. Here we introduce a prototype platform for evolving digital organisms with a complex genetic architecture. We also present preliminary results from genetic knockout experiments demonstrating that these organisms have evolved complex patterns of epistasis similar to those seen in biological organisms. We propose that digital evolution can recapitulate complex features of biological genomes in a system that is much more tractable than the biological alternatives. We hypothesize that digital organisms can help elucidate principles of the genetic architecture of complex traits and will be useful for understanding the general principles of genotype to phenotype mapping relationships.

Effect of borax on lymphocyte proliferation and sister chromatid exchange in human chromosome. *M. Pongsavee*
Department of Medical Technology, Faculty of Allied Health Sciences, Thammasat University, Pathumthani 12121, Thailand.

Borax is used as a food additive. It becomes toxic when accumulated in the body. It causes vomiting, fatigue and renal failure. The heparinized blood samples from 40 healthy Thai men were studied for the impact of borax toxicity on lymphocyte proliferation and sister chromatid exchange. The MTT assay and Sister Chromatid Exchange (SCE) technique were used in this experiment with the borax concentrations of 0.1, 0.15, 0.2, 0.3 and 0.6 mg/ml. The results showed that the numbers of cells and metaphase chromosomes were decreased when the concentrations of borax increased. The borax concentrations of 0.15, 0.2, 0.3 and 0.6 mg/ml significantly induced sister chromatid exchange in human chromosome ($P<0.05$). The borax concentration of 0.6 mg/ml had the most effectiveness to the lymphocyte proliferation and had the highest cytotoxicity index (CI). It can be concluded that borax had effects on lymphocyte proliferation and induced sister chromatid exchange in human chromosome. Toxicity of borax may lead to genetic defect in human.

Teaching genetics courses for the general public: challenges and perspectives. *M. Davis*[1,2] 1) Bryn Mawr College, Bryn Mawr, PA; 2) Wagner Free Institute of Science, Phila., PA.

The Wagner Free Institute of Science in Philadelphia has been dedicated to the mission of providing free education in the sciences for more than 150 years. The adult lecture courses in life and natural sciences are offered on an introductory college level and are open to the general public. This poster will describe the genetics education series that started in 2000.

The objectives of the genetics series are to foster understanding of basic genetics, biotechnology, and the process of scientific research. Each seven week course consists of weekly 90 minute lectures held in public libraries. Individual courses on topics including genomics, genetics in popular culture, bioterrorism, and genetics of dogs have been employed to appeal to a wide audience. College credit is not available; however optional homework assignments may be completed to earn an Institute certificate.

These community based genetics courses have presented many pedagogical challenges and rewards. Class attendees are from a diversity of socio-economic, educational, and cultural backgrounds, and they range in age from home-schooled high school students to retirees. Resources are very limited, which restricts available instructional tools and reference materials. Depth of content coverage is constantly adjusted to accommodate the different expectations of the attendees. Participation in class discussion is encouraged, resulting in a cooperative learning experience. Informal discussions are also held outside of class to enable interested attendees to pursue topics in greater detail. At the end of each course attendees complete evaluations and suggest future topics.

Surveys of attendees indicate strong interest in genetics and biotechnology. The low-key community setting promotes a collegial environment for people to ask questions about genetics. Moreover attendees interact to stimulate excitement about learning science and they seek advice on resources for self-directed learning. The Institute is always seeking new ways to fulfill its goal of making science education available to all.

Identification of essential elements and time points for mouse limb bud development. *S. Schlaubitz[1], B. C. Dawson[2], T. K. Bertin[1], E. Munivez[1], C. A. Shaw[1], B. U. Zabel[3], B. Lee[1,2]* 1) Dept. Human & Molecular Genetics, Baylor College of Medicine, Houston, TX; 2) Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX; 3) Center for Pediatrics and Adolescent Medicine, University of Freiburg, Freiburg/ Germany.

To identify genes differentially expressed in limb bud development at time points critical for limb chondrogenesis and the commencement of osteogenesis, we performed Affimetrix microarray experiments with total RNA from E11.5, E12.5, and E13.5 C57Bl/6 forelimb buds hybridized on Mouse 430 2.0 arrays. Four sets of biological replicates were normalized using RMA (Robust Multi-Array) and analysis of differential expression was performed using the software package LIMMA within the R computing environment. Differentially expressed genes of statistical relevance were grouped according their expression pattern. 707 probes were seen to be up-regulated over time with a fold change of at least 2 (p 0.01), while 768 probes were down-regulated over time (0.5 fold cutoff, p 0.01). Furthermore, we identified genes with expression profile changes over time and found 55 probes up-regulated at E12.5 in comparison to E11.5, but down-regulated at E13.5, while 293 probes showed lowest expression at E12.5 (0.5 fold and a p 0.01). Identical samples from each time point are currently being validated by quantitative real time PCR; probes with yet unknown function in mesenchymal condensation and following patterns will be further investigated. Pilot experiments revealed correlations between microarray data and validation in vitro of more than 90%. We therefore believe that our data robustly identify important genes in forelimb bud development at or near the critical time point of mesenchymal condensation.

An inherited mitochondrial DNA disruptive mutation shifts to homoplasmy in oncocytic tumor cells. *M. Lang[1], G. Gasparre[1], L. Iommarini[2], A. M. Porcelli[3], G. G. Ferri[4], I. Kurelac[1], E. Mariani[3], L. F. Pennisi[1], A. Ghelli[3], E. Bonora[1], C. Ceccarelli[5], M. Rugolo[3], N. Salfi[5], G. Romeo[1], V. Carelli[2]* 1) Unità di Genetica Medica, Policlinico Universitario S. Orsola-Malpighi, Bologna; 2) Dipartimento di Scienze Neurologiche, University of Bologna; 3) Dipartimento di Biologia Evoluzionistica Sperimentale, University of Bologna; 4) Dipartimento di Scienze Chirurgiche Specialistiche ed Anestesiologiche, Sezione di Otorinolaringoiatria, Policlinico Universitario S.Orsola-Malpighi, Bologna; 5) Unità Operativa di Anatomia e Istologia Patologica, Policlinico Universitario S. Orsola-Malpighi, Bologna.

Aim: Oncocytic tumors are characterized by cells with aberrant mitochondrial hyperplasia. Somatic mutations in mitochondrial genome (mtDNA) affecting respiratory chain complex I (CI) subunits have been previously reported in this type of neoplasia. We report the first case of inherited frameshift CI mutation in the ND5 gene associated with a specific tumor phenotype. Methods: The mtDNA was sequenced in microdissected areas from an oncocytic nasopharynx tumor and in different non-neoplastic tissues from the patient and two of his siblings in order to confirm inheritance of the mutation. Immunohistochemical analysis and western blot for CI subunits was performed on tumor tissue to study protein expression. Mutation load in mitochondria and mtDNA copy number were analyzed in all tissues. Results: The oncocytoma harbors a frameshift homoplasmic ND5 mutation which correlated with lack of expression of another mitochondrially coded CI subunit (ND6). Conversely, oncocytic cells expressing ND6 show heteroplasmy for the ND5 mutation and a *de novo* homoplasmic pathogenic ND1 mutation. Such cells also present a lower degree of mitochondrial hyperplasia as shown by mtDNA copy number. The ND5 mutation is heteroplasmic in all normal tissues of the patient and his siblings indicating a shift to homoplasmy only in the tumor. Conclusions: We conclude that complex I mutations may have a selective advantage and induce oncocytic transformation in spite of their unequivocal pathogenic effect.

A Matched Cohort from the Age Related Eye Disease Study Confirms Previous Genetic Associations with Age Related Macular Degeneration. *J. Bergeron-Sawitzke[1], B. Gold[1], S. Schlotterbeck[1], K. Visvanathan[2], R. Allikmets[3], M. Dean[1]* 1) Lab. Experimental Immunology, NCI-Frederick, Frederick, MD; 2) Bloomberg School of Public Heath, Johns Hopkins U, Dept Epi, Baltimore, MD; 3) Dept. of Ophthalmology, Columbia University, NY, NY.

Age-related macular degeneration (AMD) is a heritable late onset vision disorder. Several recent genotyping studies have established that alterations in genes in the complement cascade are associated with AMD. Complement factor H (CFH) variations have been specifically implicated as being responsible for a significant fraction of the disease. In addition, a locus at 10q26 has also contributed a genetic association with AMD. Our matched case control study confirms and extends several reported associations in and near complement related genes; it also explores the reported gene associations on chromosome 10 and with Apolipoprotein E (APOE) haplotype. Subjects were genotyped for a number of single nucleotide polymorphisms (SNPs) from CFH, Complement Component 2 (C2), Complement Component 3 (C3), Complement Factor B (CFB), ARMS2, HTRA1, and APOE. Individual SNPs, and haplotypes within proximal chromosome regions were examined for association with disease. This AREDS sample showed risk trends consistent with those seen in other population studies for allele and haplotype frequencies of CFH, C3, C2, and CFB. SNP rs10490924 on Chr. 10 in exon 1 of the ARMS2 gene showed a highly significant association with an odds ratio (OR) of 3.16 (95% CI 2.37-4.21) for the risk allele, and rs11200638 located in the proximal promoter region of HTRA1 showed a somewhat higher significant association with an OR of 3.37 (95% CI 2.50-4.57) with our AMD cases. We found that APOE haplotypes were not significantly associated with case status. Adjustments for other clinical risk factors also did not significantly alter the observed associations. This study validates the complement pathways involvement in AMD pathology and suggests that unfavorable allelic variants in complement genes have a direct role in AMD disease. These results also support previous findings that variants in the region of 10q26 exert an independent risk for AMD.

Folate pathway genes and the risk of conotruncal heart malformations. *E. Goldmuntz[1], D. Renstrom[1], S. Woyciechowski[1], P. J. Lupo[2], L. E. Mitchell[2]* 1) Division of Cardiology, Department of Pediatrics, The Childrens Hospital of Philadelphia, PA; 2) Institute of Biosciences and Technology, Texas A&M University System Health Science Center, Houston TX.

Conotruncal heart malformations (CTHM) account for 16% of all congenital heart defects (CHD), which are the most common group of major birth defects. Although CHD are prevalent and clinically significant, little is known about their etiology. Recent studies suggest that maternal folic acid supplementation may reduce the risk of CHD, and that variation in folate pathway genes may also be associated with disease risk. Studies suggest some CTHM share a common genetic basis with ventricular septal defects (VSD) and isolated aortic arch anomalies (AAA), such that these lesions were grouped together for this analysis. CTHM, VSD and AAA case-parent triads (N=727) were prospectively ascertained from the Cardiac Center at The Childrens Hospital of Philadelphia. DNA samples were genotyped for ten functional variants in nine genes involved in the folate pathway. Log-linear modeling was used to examine the associations between CTHM and both maternal and case genotype. The following case genotypes were significantly associated with disease risk: CBS 844ins68 (OR[II/IN vs. NN]=1.40 , 95% CI 1.00-1.95; p=0.05) and MTHFR A1298C (OR[AC vs. AA]=0.67, 95% CI 0.53-0.84; OR[CC vs. AA]=0.74 , 95% CI 0.50-1.12; p=0.002). In addition, maternal genotype for MTR A2756G was significantly associated with disease risk (OR[AG vs. AA]=1.40, 95% CI 1.07-1.83; OR[GG vs. AA]=1.26, 95% CI 0.69-2.29; p=0.04). This is the largest study to date on the association between genetic variation within the folate pathway and CTHM. Furthermore, this study employed a design that is robust to confounding due to population structure and allows for the evaluation of both maternal and case genotypes. The results provide additional evidence that the risk of CTHM and related cardiac defects is influenced by variation within folate pathway genes, and indicate that studies to explore these relationships further are warranted.

Ethical, legal, and economic issues arising from the application of human genome epidemiologic evidence as the basis of "personalized" patient care. *N. Markward* Pennington Biomedical Research Center, Baton Rouge, LA.

Human genome epidemiology (HuGE) is the analytical arm of public health that seeks 1) to isolate risk and protective variants that influence the distributions of diseases in populations and 2) to determine the extent to which knowledge of these relationships can be used to enhance existing disease prevention programs. In contrast, the emerging paradigm of genomic medicine (GM) is patient-centered and emphasizes the use of genomic information to personalize health care delivery and resource allocation. In concept, realization of the GM model will be achieved when patient treatment and counseling can be tailored to the specific needs of each individual based on his or her unique genomic profile. The broad objectives of HuGE and GM, as implied by these elementary descriptions, are harmonious insofar as they revolve around the altruistic notion of maximizing the health and societal benefits that can be derived from genomic exploration. However, despite this common ground, the inferential problems of population and individual risk assessment are, in fact, distinct endeavors that are guided by competing philosophical principles (utilitarianism vs. individualism) and systems of logic (deductive vs. inductive reasoning). This project examines the theoretical differences between population and individual risk; reviews the key limitations and appropriate interpretation of sample-level measures of association; and highlights important ethical, legal, and economic issues that may arise if population-based predictive tools become the *sine qua non* of GM decision-making.

Combined effects of *MC4R* and *FTO* common genetic variants on obesity in European general populations. *S. CAUCHI[1], F. STUTZMANN[1], C. PROENÇA[1], E. DURAND[1], A. POUTA[2], A. L. HARTIKAINEN[3], M. MARRE[4], S. VOL[5], T. TAMMELIN[6], J. LAITINEN[6], A. GONZALEZ-IZQUIERDO[2], A. BLAKEMORE[7], P. ELLIOTT[2], D. MEYRE[1], B. BALKAU[8], M. R. JÄRVELIN[2], P. FROGUEL[7]* 1) Genomics and Molecular Physiology, CNRS UMR8090, LILLE, France; 2) Epidemiology and Public Health, Imperial College, London, UK; 3) Obstetrics and Gynecology, University of Oulu, Finland; 4) INSERM U695, Paris, France; 5) Regional Institute for Health, La Riche, France; 6) Finnish Institute of Occupational Health, Finland; 7) Genomic Medicine, Hammersmith Hospital, Imperial College London, United Kingdom; 8) INSERM U780-IFR69, Villejuif, France.

Genome-wide association scans identified common polymorphisms in the *FTO* and *MC4R* loci that modulate body mass index (BMI) and associate with increased risk of obesity. We analyzed independent and combined effects of the *FTO* rs1421085 and the *MC4R* rs17782313 risk alleles on BMI, prevalence and incidence of obesity, fat mass, and their interactions with physical activity levels and gender in two European prospective population-based cohorts of 4,802 Finnish adolescents (NFBC 1986) and 3,167 French adults (DESIR). Compared to participants carrying neither *FTO* nor *MC4R* risk allele (20-24% of the populations), subjects with 3 or 4 risk alleles (7-10% of the populations) had a 3-fold increased susceptibility of developing obesity during childhood. In adults, their combined effects were more modest (~1.8-fold increased risk) and associated with a 1.3% increase in fat mass (P=0.001). Prospectively, we demonstrated that each *FTO* and *MC4R* risk allele was a predictive factor of obesity development, associated with a 24% increased incidence (P=0.02). The Z-BMI and ponderal index of newborns homozygous for the rs1421085 C allele were 0.1 units (P=0.02) and 0.3g/cm^3 (P=0.005) higher, respectively, than in those without *FTO* risk allele. The *MC4R* rs17782313 C allele was more associated with obesity and fat mass deposition in males than in females (P=0.003 and P=0.03, respectively) and low physical activity was found to accentuate the effect of the *FTO* polymorphism on BMI increase and obesity prevalence (P=0.008 and P=0.01, respectively).

Mutational spectrum of the Oral-facial-digital type I syndrome: a study on a large collection of patients. *B. Franco[1,2], C. Prattichizzo[1], M. Macca[1,2], V. Novelli[1], R. Tammaro[1], G. Giorgio[1], A. Barra[1], The OFDI collaborative group* 1) Telethon Institute of Genetics and Medicine (TIGEM), Fondazione Telethon, Naples, Italy; 2) Medical Genetics Services, Department of Pediatrics, Federico II, University of Naples.

Oral-facial-digital type I (OFDI; MIM 311200) syndrome is a male-lethal X-linked dominant developmental disorder belonging to the heterogeneous group of Oral-facial-digital syndromes (OFDS). OFD type I is characterized by malformations of the face, oral cavity and digits. CNS abnormalities and cystic kidney disease can also be part of this condition. This rare genetic disorder is due to mutations in the OFD1 gene (MIM# 300170) that encodes a centrosome/basal body protein necessary for primary cilium assembly and for left-right axis determination, thus ascribing OFDI to the growing number of disorders associated to ciliary dysfunction. We now report a mutation analysis study in a cohort of 109 unrelated affected individuals collected worldwide. Putative disease-causing mutations were identified in about 80% of patients. We describe 67 different mutations, 64 of which represent novel mutations, including 36 frameshift, 9 missense, 11 splice-site and 11 nonsense mutations. Most of them concentrate in exons 3, 8, 9, 12, 13 and 16, suggesting that these exons may represent mutational hotspots. Phenotypic characterization of the patients collected provided a better definition of the clinical features of OFD type I syndrome. Differently to what previously observed, our results indicate that renal cystic disease is present in 60% of cases with over 18 years of age. Genotype-phenotype correlation did not reveal significant associations apart for the high-arched/cleft palate most frequently associated to missense and splice-site mutations. Our results contribute to further expand our knowledge on the molecular basis of OFD type I syndrome. In addition these results will help in further defining the clinical spectrum and recognition of Oral-facial-digital syndromes.

Periventricular heterotopia, mental retardation and epilepsy associated with 5q14.3-q15 deletion. *C. Cardoso[1], A. Boys[2], E. Parrini[3], A. Carabalona[1], S. Khantane[1], C. Mignon-Ravix[4], J. M. McMahon[5], E. Bertini[6], F. Novara[8], O. Zuffardi[8], L. Villard[4], S. Giglio[3], B. Chabrol[7], H. R. Slater[2], A. Moncla[7], Y. Ben-Ari[1], I. E. Scheffer[5], A. Represa[1], R. Guerrini[3]* 1) INMED, INSERM U901, Université de la Méditerranée, Marseille, France; 2) Victorian Clinical Genetics Services, Melbourne, Australia; 3) Children's Hospital A. Meyer, University of Florence, Italy; 4) INSERM U910, Faculté de Medecine La Timone, Marseille, France; 5) Austin Health and Royal Childrens Hospital, Melbourne, Australia; 6) Bambino Gesù Hospital, Rome, Italy; 7) Timone Childrens Hospital, Marseille, France; 8) Genetica Medica, Università di Pavia, Italy.

Periventricular heterotopia (PH) is an etiologically heterogeneous disorder characterized by nodules of neurons ectopically placed along the lateral ventricles. Most affected patients have seizures and their cognitive level varies from normal to severely impaired. To date, two genes have been identified to cause PH. Mutations in FLNA (Xq28) and ARFGEF2 (20q13) are responsible for X-linked bilateral PH and a rare autosomal recessive form of PH with microcephaly. Eleven additional distinct anatomoclinical PH syndromes, including chromosomal rearrangements involving the 1p36, 5p15 and 7q11 regions, have also been reported but the genes implicated remain unknown. Here, we report the clinical and imaging features of three unrelated patients with epilepsy, mental retardation and bilateral PH in the walls of the temporal horns of the lateral ventricles, associated with a de novo deletion of the 5q14.3-q15 region. Using CGH arrays and FISH analysis, we defined the boundaries of the deletions. The three patients shared a common deleted region spanning 5.8 Mb and containing 14 candidate genes. To identify the PH-associated gene, we are currently screening these genes for mutations in sporadic cases with PH. We are also using the in utero RNA interference approach to identify if one of these candidate genes contributes to neuronal migration. This combined strategy should allow us to identify the gene that is implicated in the temporal PH and provide new insights into the genetic and developmental basis of this cortical malformation.

Identification of a risk haplotype within PSORS4 locus. *C. Sinibaldi[1], V. Foti Cuzzola[1,2], C. Peconi[1], A. M. Mazzotta[3], P. Amerio[4], S. Chimenti[3], P. Bramanti[2], J. Fischer[5], E. Giardina[1,6], G. Novelli[1,7,8]* 1) Department of Biopathology and Centre of Excellence for Genomic Risk Assessment in Multifactorial and Complex Diseases School of Medicine, Tor Vergata University of Rome, Italy; 2) IRCCS Neurolesi Center Bonino-Pulejo, University of Messina, Italy; 3) Department of Dermatology, Tor Vergata University, Rome, Italy; 4) Department of Dermatology, University of Chieti, Italy; 5) Centre National de Génotypage, EVRY, France; 6) Univerisy Carlo BOof Urbino, Italy; 7) San Peter Hospital, Fatebenefratelli, Rome, Italy; 8) Department of Cardiovascular Medicine, University of Arkansas for Medical Sciences, Little Rock, AR,USA.

Psoriasis (PS) is a chronic inflammatory skin disorders triggered by both genetic and environmental factors. A susceptibility locus mapped on chromosome 1q21 have been identified for the disease (PSORS4). We refined the PSORS4 susceptibility locus using a LD approach in a preliminary cohort of 128 PS trios. Within the redefined region is a single gene (*LOR*) encoding for loricrin, the major component of the epidermal cornified envelope comprising about 70-85% of the total protein mass of the cornified. Although *LOR* was a good positional and functional candidate gene we failed to reveal evidence of association for intragenic SNPs. To disclose the identity the susceptibly variants we performed an association study by typing a dense panel of SNPs within PSORS4. By typing both familial (128 trios) and sporadic samples (400 psoriatic patients and 400 healthy controls) we identified a conserved LD block (38kb long) upstream LOR gene on risk chromosomes. Thus we performed a re-sequencing of the entire LD block in our patients to identify recombinant haplotypes and refine the locus. Our preliminary results suggested a putative role of specific SNPs for the control of expression of psoriasis misregulated genes within EDC such *LOR, S100A8, S100A9, LELP1* and some SPRRs. Acknowledgements This work was supported by A.DI.PSO.

Knock-In Mouse Model of Dilated Cardiomyopathy Caused by Titin Mutation. *B. Gerull[1], M. Gramlich[1], B. Michely[1], C. Krohne[2], I. Morano[1], H. Granzier[3], S. Labeit[2], L. Thierfelder[1]* 1) Max-Delbrueck-Center for Molecular Medicine, Berlin, Germany; 2) Anesthesiology and Intensive Operative Medicine, University Hospital, Mannheim, Germany; 3) VCAPP, Washington State University, Pullmann, USA.

Dilated cardiomyopathy (DCM) is the most common form of primary myocardial diseases and the third most common cause of heart failure. Familial occurrence, mostly as an autosomal dominant trait, is responsible for 20-30% of all DCM cases. We have previously shown that mutations in the giant muscle filament titin (TTN) cause dilated cardiomyopathy. In a large DCM kindred (A1) with autosomal dominant inherited DCM, we could identify a 2 bp insertion mutation in exon 326 of TTN. This heterozygous nonsense mutation leads to a framshift generating a premature stop codon. We have recently evaluated a cardiac biopsy sample from an affected family member of kindred A1 showing that no truncated protein is observed in western blot analysis. To further investigate the functional consequences of the identified human TTN mutation, we generated a mouse model that includes the 2bp insertion mutation at the corresponding site in the mouse genome. Heterozygous mice are viable and fertile. As in the human situation, the truncated titin is not detectable in western blot analysis of cardiac tissue indicating haploinsufficiency. The ventricles of the heterozygous animals show a decrease in ventricular stiffness as seen in isolated working heart pressure measurements and transmitral Doppler echocardiography (E:A 1.34 vs. 1.075, $p<0.01$; IVRT 13.57ms vs. 17.01ms, $p<0.05$). When exposed to angiotensin II (1.4 mg/kg/d for 14d) as a cardiac stressor, heterozygous animals develop dilatation of the left ventricles (4.45mm vs. 3.77mm, $p<0.05$) with impaired fractional shortening (25.12% vs. 32.86%, $p<0.01$) and a diffuse myocardial fibrosis recapitulating features of patients with DCM. Homozygous mutant embryos die before E9.5 as a result of unformed sarcomeres observed in electronmicroscopy. Our mouse model shows that a mutation in TTN leads to impaired biomechanical properties of the heart, resulting in left ventricular dilatation and decreased systolic function, thereby recapitulating the human phenotype.

Multiple Displacement Amplification (MDA) in forensic samples: time for reconsideration. *I. Pietrangeli[1], C. Martone[1], E. Giardina[1], S. Zampatti[1], P. Marsala[2], L. Gabriele[2], C. Pipolo[2], O. Ricci[2], G. Solla[2], A. Spinella[2], G. Novelli[1,3]* 1) Centre of Excellence for Genomic Risk Assessment in Multifactorial and Complex Diseases, School of Medicine, Tor Vergata University of Rome, Italy; 2) Direzione Centrale Anticrimine, Servizio di Polizia Scientifica, Rome, Italy; 3) Division of Cardiovascular Medicine, Department of Medicine, University of Arkansas for Medical Sciences, Little Rock, AR 72205.

Whole genome amplification (WGA) is a technique developed for genetic analysis to obtain sufficient amount of DNA from small pools of cells or even single cells. Presently, the usefulness of WGA in STR-based forensic analysis is limited because of allelic dropout (ADO) and bias in peak area ratios observed in low copy number (LCN) templates. Typing of SNPs is more effective than STRs in LCN templates and less prone to ADO when WGA is applied. We recently validated a panel of 26 TaqMan SNP assays selected to show an high sensibility in LCN templates. In this work we evaluated the performance of multiple displacement amplification (MDA) applied to our optimized SNP assays for typing 300 LCN (1 ng, 0.1 ng and 0.01 ng) and 10 severely degraded DNA samples. We observed an higher positive typing rate in amplified DNA respect to genomic DNA and 100% of concordance rate between amplified vs control DNA for 1 ng and 0.1 ng dilutions. Samples of 0.01 ng revealed a lower concordance rate (99.34%) due to the failure of amplification of one allele in heterozygous samples (ADO). In order to improve the concordance rate in 0.01 ng dilution we applied an optimized TaqMan Genotyping Master Mix to our SNPs panel. ADO occurrence was significantly reduced by using the optimized protocol resulting in better concordance rate even in 0.01 ng samples (99.93%). Significant results have been also observed in artificially degraded DNA and single cell samples. Finally, we successfully typed challenging DNA samples for current STR forensic protocols. These results showed that MDA should be considered as a suitable option for specifically designed SNP assays in challenging forensic caseworks. Work supported by EU FP6 projects NACBO (contract no. NMP4-CT-2004-500804).

Phenotypic consequences of gene CNV: uncoupling gene dosage from positional effect. Clues from the Smith-Magenis (SMS) and Potocki-Lupski (PTLS) syndromes mouse models. *K. Walz[1], J. Molina[1], J. R. Lupski[2]* 1) Region de los Rios, Centro de Estudios Cientificos, Valdivia, Chile; 2) Human Molecular Genetics Department, Baylor College of Medicine, Houston, Tx, USA.

Genomic disorders result from recurrent DNA rearrangements involving unstable genomic regions, and are frequent diseases (~1 per 1,000 births). DNA rearrangements such as deletions, duplications or inversions, can cause genomic disorders. In a subset of such conditions the rearrangements comprise multiple unrelated contiguous genes that are physically linked and thus have been referred to as contiguous gene syndromes (CGS). Smith Magenis syndrome (SMS; MIM182290) is a CGS associated with a microdeletion within chromosome 17 band p11.2. Human chromosome 17p11.2 is syntenic to the 32-34 cM region of murine chromosome 11. The number and order of the genes are highly conserved. By chromosome engineering we have generated mouse models for SMS (Df(11)17/+) and Potocki-Lupski syndrome (PTLS; MIM610883) (Dp(11)17/+). Both mouse models recapitulate several aspects of the patients clinical presentation. To analyze the relationship between genome structural changes and phenotypes we study these mouse models.This is a unique opportunity to uncouple the phenotypes that are due to gene copy number variation versus potential positional effect of the rearrangement. In order to do so we studied the phenotypic consequences of restoring the correct gene copy number within the interval, albeit in a uniallelic fashion, Df(11)/17/Dp(11)17 mice. Viability, weight differences, craniofacial abnormalities, activity levels, anxiety behaviors, memory and learning, and social behaviors were studied among others in mice that carried a deletion (Df(11)17/+), a duplication (Dp(11)17/+) or were compound heterozygous (Df(11)/17/Dp(11)17) in a C57BL/6-Tyrc-Brd inbred genetic background to minimize the effect of genetic background in the analysis. We found that gene copy number restoration was not able to rescue all phenotypic manifestations, highlighting the delicacy of genomic control mechanisms, and uncoupling, for the first time, the effects of gene CNV and genomic structural changes.

Detection of known and novel mutations involved in Retinitis Pigmentosa in a cohort of Northern Irish patients using resequencing genechip technology. *G. R. Clark, D. Muszynska, P. Crowe, G. J. McKay, S. Alexander, J. O'Neill, G. Silvestri, D. A. Simpson* Centre for Vision Science, Queens University Belfast, UK.

The inherited retinal degenerative disease Retinitis Pigmentosa (RP) is extremely genetically heterogeneous. With over 20 genes implicated in non-syndromic recessive RP to date, it is very difficult to identify which gene is mutated in a specific patient. We have therefore developed a microarray capable of resequencing reported mutations and the exons within which they are found. A database of all reported mutations which cause recessive or X-linked RP was compiled and an Affymetrix customseq resequencing array designed to screen these and adjacent sequences. In total 30 kb from 22 genes were tiled on the array. Approximately 100 amplicons spanning the regions of interest were amplified from each patient DNA sample. An average call rate of 91% was achieved for all the sequences analysed in a cohort of 30 recessive and 35 sporadic RP patients. A total of 16 previously reported SNPs and 10 known mutations in CRB1, RGR, RPGR, RPE65 & USH2A were identified. Seventeen novel sequence variants were screened in 350 ethnically matched population controls, using the Sequenom MassARRAY genotyping method. Of these, 8 are likely to be novel SNPs whilst 9 were not observed in the controls and therefore represent likely novel mutations. The segregation of the known and putative novel mutations was assessed in additional family members using direct DNA sequencing. This study has demonstrated that this resequencing platform can provide a rapid and effective screen for RP mutations and provides a new tool to include in screening strategies. Technological improvements will facilitate fuller coverage of recently discovered genes in subsequent genechip designs, although PCR amplification remains a limiting factor. It is anticipated that the improved detection of RP mutations will facilitate genotype:phenotype correlations, better prognosis and application of therapeutic interventions such as gene therapy.

High resolution array comparative genome hybridization reveals de novo and rare inherited changes in patients with congenital diaphragmatic hernia. *M. J. Wat[1], A. M. Holder[1], C. J. Fernandes[2], A. Johnson[3], K. P. Lally[4], D. Tibboel[6], A. de Klein[5], B. Lee[1,7], D. A. Scott[1]* 1) Molecular & Human Genetics; 2) Pediatrics; 3) Ob & Gyn, Baylor College of Medicine, Houston, TX; 4) Ped Surgery, U of Texas Medical School, Houston, TX; 5) Clinical Genetics; 6) Paediatric Surgery, Erasmus Medical Center, Rotterdam, The Netherlands; 7) Howard Hughes Medical Institute.

Congenital diaphragmatic hernia (CDH) is a relatively common sporadic birth defect, with an incidence of ~1:3,000 live births. We are using a positional candidate strategy based on chromosomal data to localize and identify genes that cause or predispose to the development of CDH. By reviewing published reports, we identified 19 chromosomal regions that are recurrently deleted or duplicated in CDH. We hypothesize that each of these regions harbors one or more CDH-related genes. To identify additional CDH-related regionsand refine those previously describedwe screened a cohort of CDH patients using a combination of high density genome-wide array comparative genome hybridization and quantitative PCR. Using this combined approach we identified over 45 rare genomic variants. These included a ~700kb *de novo* deletion of 16p11, a region which has not been previously associated with CDH. Other *de novo* changes identified in our cohort offer insight into the spectrum of diaphragmatic defects and related anomalies associated with deletions of *FOG2* and other genes on chromosome 8q22 and provide further evidence that up-regulation of genes on chromosome 11q23-24 and 13q12 predispose to the development of CDH. Rare inherited changes identified in this cohort involved several signaling pathway genes that regulate cell proliferation, differentiation, and/or migration. One of the most intriguing changes is a 500kb paternally-inherited deletion involving *MAP2K5*. *MAP2K5* is essential for normal embryonic development but homozygous *Map2k5* null mice die before its role in diaphragm development can be determined. It is possible that a reduction in *MAP2K5* expression, in combination with other genetic/environmental stressors, led to the development of right-sided CDH in this child.

Expression profiling of subcutaneous and visceral adipose tissue in lean vs. obese humans. *M. Hubal[1], E. Hoffman[1], H. Barakat[2]* 1) Research Center for Genetic Medicine, Children's National Medical Center, Washington, DC; 2) The Diabetes and Obesity Center, Brody School of Medicine, East Carolina University, Greenville NC.

Evidence shows altered metabolism in adipose tissue of obese subjects that are matched for age and sex to lean controls. The molecular mechanisms driving these differences have not been defined. Furthermore, it is not yet known whether different adipose tissue compartments exhibit similar obesity-driven changes at the molecular level. Our aim was to use expression profiling to identify differences in transcription in two distinct adipose tissue depots from lean (L) and obese (Ob) humans. Visceral (VF) and subcutaneous (SF) adipose samples were collected from female morbidly obese (N=4) and lean controls (N=5). RNA was hybridized to Affymetrix microarrays. A repeated measure ANOVA (group * tissue; $p<0.01$) was used for analysis. Obesity up- or down-regulated the expression of 1687 genes in SF and 937 genes in VF samples. Of these genes, 93 genes were similarly affected by obesity in both depots, including defects in several energy metabolism enzymes including dihydrolipoamide branched chain transacylase E2 (DBT; Ob/L ratio of -1.6 in both SF and VF) and succinate dehydrogenase complex, subunit D (SDHD; Ob/L ratio of -2.6 in SF and -1.3 in VF). Lean individuals demonstrated more genes with differential expression between depots (4811 vs. 267) than the obese group, many of which map to the NF-B canonical pathway. In the L group, expression of NF-B pathway components was generally higher in VF than SF (11 genes higher in VF including IL1, TNFR and AKT3), while these genes were similarly expressed in SF and VF in the obese group. These findings suggest obese individuals have impaired expression of enzymes related to energy metabolism in both subcutaneous and visceral adipose. A high degree of tissue-specificity in the lean group was seen in the NF-B pathway with higher expression in VF, an effect that was absent in the obese group, suggesting a loss in normal expression in obese subcutaneous adipose tissue, which could exacerbate inflammatory and cell proliferation signals in obese individuals.

Resequencing of FLG gene in atopic dermatitis. *R. Cascella[1], C. Sinibaldi[1], C. Peconi[1], N. Paolillo[1,2], E. Galli[3], L. Chini[4], V. Moschese[4], P. Rossi[4,5], E. Giardina[1,6], G. Novelli[1,3,7]* 1) Department of Biopathology and Centre of Excellence for Genomic Risk Assessment in Multifactorial and Complex Diseases, School of Medicine, Tor Vergata University of Rome, Italy; 2) Neuropharmacology "Mondino Center" -Tor Vergata University of Rome, Italy; 3) San Pietro Hospital, Fatebenefratelli, Rome, Italy; 4) Department of Pediatrics, Tor Vergata University, Rome, Italy; 5) Division of Immunology and Infectious Disease, Department of Pediatrics, Childrens Hospital Bambino Gesù, Rome, Italy; 6) University Carlo Bo of Urbino, Italy; 7) Department of Cardiovascular Medicine, University of Ankansas for Medical Sciences, Little Rock, AR, USA.

Atopic dermatitis (ATOD) is a frequent chronic inflammatory skin disease associated with immunologic and epidermal abnormalities triggered by both genetic and environmental factors. Linkage and association analysis revealed a susceptibility locus on chromosome 1q21 (ATOD2) and we refined the susceptibility locus within a region of 42 kb upstream the *LOR* gene. Recently, it has been reported that common mutations (R501X and 2282del4) within the filaggrin (*FLG*) gene, located 959 kb from *LOR*, are associated with ATOD in a number of populations. We recently failed to confirm the association of these mutations in italian population suggesting the existence of population-specific mutations or else a negative selection of *FLG* mutations. To verify the presence of unknown mutations here we report a mutational analysis of *FLG* based on the full re-sequencing of the entire gene in an independent cohort of 100 Italian ATOD patients. Although several unknown SNPs have been identified, preliminary data confirmed the low frequency of common *FLG* mutations and excluded the presence of other mutations. Taken together these results may suggest that *FLG* is not the ATOD2 gene in Italian population. Acknowledgements Work funded by the Italian Ministry of Health.

Phenotypically homogeneous autism families yields evidence for epistasis between engrailed 2 and loci on 13q13 and 13q14. *C. W. Bartlett[1], P. Garavito[2], N. Gharani[2], M. A. Azaro[2], J. F. Flax[2], O. Stein[1], R. Goedken[1], E. Di-Cicco Bloom[3], J. H. Millonig[3], V. J. Vieland[1], L. M. Brzustowicz[2]* 1) Battelle Ctr Mathematical Med, The Research Institute at Nationwide Children's, Columbus, OH; 2) Department of Genetics, Rutgers University, Piscataway, NJ; 3) Center for Advanced Biotechnology and Medicine; Department of Neuroscience and Cell Biology, University of Medicine and Dentistry of New Jersey, Piscataway, NJ.

Autism, a genetically complex developmental disorder, has been extensively studied by genome-wide linkage analysis and candidate gene association studies. Results from initial studies were difficult to replicate/interpret across research groups, and neither increased sample size alone nor GWAS has proved to be a panacea. Here we describe a complementary approach based on careful selection of a clinically homogeneous set of families. Using families from the Autism Genetic Resource Exchange, we performed extensive clinical filtering of families based on diagnostic, medical and medical genetic criteria. When the study began, a total of 102 families meeting our strict inclusion criteria had Affymetrix 10k SNP data available (posted to the AGRE website by the Autism Genome Project). Genome-wide linkage analysis using the posterior probability of linkage (PPL) framework yielded evidence for 13q14 (PPL=36%) and 15q13 (PPL=25%). We then conducted two-locus epistasis analysis using the known risk haplotype in engrailed 2 as bait, producing an additional locus of interest on 13q13 (PPL=50%) and further strengthened the evidence for 13q14 (PPL=47% up from 36%). Subsequently Affymetrix 500k data on 215 additional families meeting our criteria became available; we sequentially updated our results to accumulate linkage evidence across the two datasets. Evidence for 13q14 increased to 64% with additional epistasis analysis ongoing. While our follow-up association analysis using htSNPs genotyped by our group did not yield compelling evidence for LD (using PPLD), other groups have implicated all 3 of our loci. In summary, based on a small group of very carefully selected families, we have obtained compelling evidence of linkage to 3 loci as well as evidence of epistasis.

Interpretation of genetic association studies: Markers with widely replicated highly significant odds ratio may be poor classifiers. *J. Jakobsdottir[1], M. B. Gorin[2], Y. P. Conley[3,4], R. E. Ferrell[4], D. E. Weeks[1,4]* 1) Department of Biostatistics, Graduate School of Public Health, University of Pittsburgh, PA, USA; 2) Department of Ophthalmology and Jules Stein Eye Institute, The David Geffen School of Medicine, UCLA, CA, USA; 3) Department of Health Promotion and Development, School of Nursing, University of Pittsburgh, PA, USA; 4) Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, PA, USA.

Recent successes in the discoveries of potentially causal SNPs for complex diseases hold great promise and commercialization of genomics in personalized medicine has already begun. The hope is that genetic testing will benefit patients and their families and encourage positive life-style changes and guide clinical decisions. However, for many complex diseases, such as age-related maculopathy (ARM), it is arguable whether the era of genomics in personalized medicine is here yet. We discuss and explore some of the issues geneticists and physicians face when presenting the individual-level risk calculations. Our focus will be the clinical validity and utility of genetic testing with additional emphasis on two popular statistical methods for evaluating markers. The majority of genetic association studies are etiological studies aimed at finding variants strongly correlated with disease risk. We then hope to use these variants in individual-level risk estimation, classification, and clinical decision-making. Using our ARM data, we examine the validity of the three strongest risk SNPs discovered so far. For example by using an additive model of the *CFH*, *LOC387715*, and *C2* variants, with odds ratios (ORs) 2.9, 3.4, 0.4, and P-values 10^{-13}, 10^{-13}, 10^{-3}, respectively, the area under the receiver operating characteristic curve is 0.79 but the positive predictive values (PPVs) assuming prevalence of 15%, 5.5%, and 1.5% (which are realistic for age groups 80, 65, and 40 years and older) are only 30%, 12%, and 3%, respectively. PPV is the probability of disease given genotypes and therefore a more relevant risk estimator than the OR.

Mapping of the *blind sterile 2 (bs2)* locus in mice. *R. P. Liegel[1], B. Chang[2], D. J. Sidjanin[1,3]* 1) Cell Biology, Medical College of Wisconsin, Milwaukee, WI; 2) The Jackson Laboratories, Bar Harbor, ME; 3) The Human and Molecular Genetics Center, Medical College of Wisconsin, Milwaukee, WI.

Blind sterile locus 2 (bs2) is a novel autosomal recessive mouse mutation that arose spontaneously on C57BL/6 background. *bs2* mice exhibit a phenotype of congenital cataracts as well as male-specific sterility. The goal of this study is to identify the gene containing the mutation responsible for the *bs2* phenotype, as well as to investigate the human ortholog of the *bs2* locus. In order to map the *bs2* locus, *bs2* homozygote females were outcrossed to the Cast/Ei strain followed by F1 *(bs2 X Cast/Ei)* intercross to generate F2 progeny. At three weeks of age, progeny were phenotyped and then euthanized. Eye and testis tissues were collected for genetic and histological analysis. Clinically, *bs2* homozygote mice exhibit bilateral nuclear cataracts and micropthalmia. Histological evaluation of eye sections shows that *bs2* mice have severely disrupted lens epithelial and fibroblast cells resulting in mature cataracts. Histological evaluation of testes show that *bs2* testes are smaller than WT controls, do not contain mature sperm, and contain large multinucleate cells in the seminiforous tubules. Linkage analysis mapped *bs2* to mouse chromosome 2, approximately 45cM from the centromere. Evaluation of the Mouse and Human Genome databases did not yield any obvious candidate genes responsible for both cataract and spermatogenesis phenotypes. Our current hypothesis is that the *bs2* mouse phenotype results from a mutation in a novel gene essential for lens transparency and male fertility. However, a possibility exists that *bs2* allele is due to a large deletion encompassing two independent genes responsible for the eye and testis phenotype. Further studies are needed to establish the onset and progression of both the eye and testis phenotypes in *bs2* mice; immunohistochemical staining is currently underway to characterize lens fiber maturation defects. In addition, we are currently focusing our efforts to narrow the critical region containing the mutation via microsatellite and SNP mapping to facilitate identification and sequencing of the gene(s) responsible for the *bs2* phenotype.

Genotype imputation accuracy across worldwide human populations. *L. Huang[1], L. Yun[1], A. B. Singleton[2], J. A. Hardy[3], G. Abecasis[1], N. A. Rosenberg[1,4], P. Scheet[1]* 1) Department of Biostatistics, University of Michigan; 2) Laboratory of Neurogenetics, National Institute on Aging; 3) Institute of Neurology, University College London; 4) Department of Human Genetics, University of Michigan.

A current approach to mapping complex disease susceptibility loci in genome-wide association (GWA) studies is to leverage the information in a reference database of dense genotype data. By modeling the patterns of linkage disequilibrium (LD) in a reference panel, such as HapMap, and by applying the fitted model to genotypes obtained from study samples (such as those from a case-control study), genotypes that are not directly measured in the samples can be imputed and tested for association. This strategy has been successful for GWA studies involving populations that are well-represented by existing high-density reference panels. We used genotypes at 512,762 autosomal SNP loci in 443 unrelated individuals from 29 populations worldwide from the Human Genome Diversity Project (HGDP) to evaluate the "portability" of the HapMap reference panels for imputation in studies of diverse populations. When leveraging a single HapMap panel to impute randomly masked genotypes, European populations had the highest imputation accuracy, followed by populations from East Asia, Central/South Asia, the Americas, Oceania, the Middle East, and Africa. For each population, we also interrogated optimal mixtures of reference panels that produced the maximal imputation accuracy. From an independent survey of the same samples, in which additional 1008 SNPs were typed, we evaluated the imputation accuracy in a scenario in which all the genotypes at a given SNP position were unobserved and were imputed based on SNP data available from a commercial "SNP chip." In this setting, we obtained similar results to those produced when masking genotypes randomly. Our results can serve as a guide for selecting appropriate reference panels for analysis of GWA data. In addition, this study helps characterize genetic distances among human groups in terms of a highly practical metric -- imputation accuracy.

Two novel EBP mutations in Conradi-Hünermann-Happle syndrome. *S. Ausavarat[1], P. Tanpaiboon[2], S. Tongkobpetch[1], K. Suphapeetiporn[1], V. Shotelersuk[1]* 1) Division of Medical Genetics and Metabolism, Department of Pediatrics, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand; 2) Department of Pediatrics, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand.

Conradi-Hünermann-Happle syndrome, also known as chondrodysplasia punctata type 2 (CDPX2), is an X-linked dominant disorder characterized by skin defects, skeletal and ocular abnormalities. CDPX2 was shown to be caused by mutations in the gene encoding emopamil binding protein (EBP). At least 58 different mutations have been described. Here we present clinical and molecular findings in two unrelated Thai girls with CDPX2. Mutation analysis by PCR-sequencing the entire coding region of EBP successfully revealed two potentially pathogenic, novel mutations, c.616G>T and c.382delC. This study has expanded the spectrum of the EBP gene mutations causing CDPX2.

The role of geography and drift in human adaptation. *G. Coop[1,2], J. K. Pickrell[1], S. Kudaravalli[1], J. Novembre[1,3], R. M. Myers[4], M. W. Feldman[5], J. K. Pritchard[1]* 1) Dept Human Genetics, University of Chicago, Chicago, IL; 2) Evolution and Ecology Section, University of California Davis. CA; 3) Ecology and Evolution Department, UCLA. CA; 4) Department of Genetics, Stanford University. CA; 5) Department of Biological Sciences, Stanford University. CA.

Several types of observations argue for a role of adaptation in recent human evolution, including selection signals at candidate genes and genome-wide selection scans. Nonetheless, using genome-wide SNP data from the HapMap, Perlegen, and Human Genome Diversity Panel studies, we find evidence that strong sustained selection is extremely rare in recent human evolution (the last ~70,000 years). There are very few fixed, or nearly fixed differences between human populations, and most fixation events have occurred in the populations that show the most drift at neutral loci. Moreover, the geographic distribution of putatively selected alleles almost invariably conforms to population clusters identified using randomly chosen genetic markers; this indicates that selected alleles have rarely spread across barriers to neutral gene flow. In summary, we propose that the geographic distribution of favored alleles is largely determined by population history and migration, and that the geographic distribution of SNPs with extreme Fst is best described by a model of relatively weak selection with genetic drift. When humans adapt to new environments it may often be via modest allele frequency changes in multiple genes simultaneously.

Amplification and elevated expression/activity of AKT3 frequently occurs in hepatocellular carcinomas and is implicated in the molecular pathogenesis of liver cancer. *J. Xu[1], J. Pei[1], D. Koumbi[1], A. N. Zekri[2], J. R. Testa[1]* 1) Human Genetics Program, Fox Chase Cancer Center, Philadelphia, PA; 2) National Cancer Institute, Cairo University, Cairo, Egypt.

Overexpression and activation of AKT have been reported as important events in the promotion, recurrence, prognosis, and metastasis of hepatocellular carcinoma. The AKT3 gene locus at 1q44 has been shown to be amplified in a majority of clinical samples of human hepatocullular carcinoma. Here we report that the AKT3 locus was amplified in both clinical samples and cell lines of hepatocellular carcinoma by using Affymetrix single nucleotide polymorphism (SNP) mapping arrays. Amplification was verified by real-time PCR analysis of genomic DNA by using primers specific for AKT3. Copy number for the AKT3 gene ranged from 2.5 to 5. Further analysis found that AKT3 protein was also highly expressed and activated in samples exhibiting the gene amplification. Knockdown of AKT3 in hepatocellular carcinoma cells resulted in diminished cell viability. These findings suggest that the amplification of AKT3 may contribute to elevated expression and activity of the AKT3 protein, which may play a significant role in the molecular pathogenesis of hepatocellular carcinoma.

Detection and Control of Bias in Genome-wide Association Studies: A Systematic Review. *T. Pearson*[1, 2], *T. Manolio*[2] 1) Community and Preventive Medicine, University of Rochester Medical Center, Rochester, NY; 2) Office of Population Genomics, NHGRI, NIH, Bethesda, MD.

The heterogeneity of results from gene association studies has several possible explanations, one of which is bias in subject selection and in collection of data on genotype and phenotype. To study the potential for bias and its control in genome-wide association studies (GWAS), we performed a systematic review of the first 109 GWAS entered into the NHGRI Online Catalog of GWAS (http://www.genome.gov/gwastudies). These studies examined 91 discrete disease traits and 40 quantitative traits; 71% had a case-control design. Assessment and control of potential genotyping errors included genotyping completion rates in 75% of studies; 80% performed tests to estimate genotyping error; 56% assayed multiple samples for quality control; and 76% used methods to control for population stratification. Information on phenotype definition and the selection of study subjects was less frequent, however. The method of definition of phenotype was provided in 67% by primary reports, 28% by online supplements, and not at all in 5%. Only 36% of discovery studies used population-based cases or controls. A minority of reports (33%) presented tables comparing cases and controls for potential confounding, and 3.7% tested differences for statistical significance. Only 21% of results were adjusted for baseline differences between cases and controls; analyses stratified by potential confounders in 24% of studies. Nonresponse rates could be assessed only in the 9.2% of reports which published participation rates; only one report compared characteristics of study participants and nonparticipants. In conclusion, the literature pertaining to GWAS has emphasized quality control of genomic analysis and genotyping. The design, conduct, and presentation of GWAS has been less consistent in quality control of phenotype description and in the avoidance and control of potential biases of selection and description of study subjects.

Expression profile of DNA damage signaling and repair responsive genes in Alzheimers disease patients. *D. V. N. P. Oliveira[1,3], D. J. Xavier[1], J. C. Moriguti[2], E. T. Sakamoto-Hojo[1], Oliveira* 1) Genetics Department, Faculty of Medicine of Ribeirão Preto, São Paulo University; 2) Geriatrics Department, Medical Clinic HCFMRP/USP; 3) Radiation Biology Center, Kyoto University, Kyoto, Japan.

Alzheimer's disease (AD) is a chronic neurodegenerative disorder with an impact on public health. The identification of risk factors (genetic and protective factors) related to AD has become of great importance. Among them, there is evidence that the development of AD is strictly related to oxidative damage. Therefore, this work laid in two main lines: (1) evaluating some gene expression levels, by Real Time PCR, associated to oxidative DNA damage responsive system, such as other DNA damage responsive genes, and also those that there might be potentially related to the disease; and (2) a general gene expression screening, by cDNA microarrays. Both experiments using AD patients and healthy donors lymphocytes. Curiously, it was found that all genes analyzed by Real Time PCR, with exception of APOE (FC=+1.43), were downregulated, compared to healthy donors, as well as those genes that do not have yet been reported to be linked with the disease, including *PRKDC*, *FEN1*, *ATM* and *ATR*. Additionally, it was also found 43 differently modulated genes by cDNA microarrays (FDR0.06). Such data showed that, not only oxidative damage responsive system might play a role in the development of AD, but also other DNA damage signalling and repair systems might as well, whether they are a cause or a consequence of that. Besides, the modulation of genes associated to the -amyloid anabolic pathway also indicated its relationship with the pathology. Concluding, those results pointed out that the transcriptional profiles observed among AD patients indicate a multiple and complex signalling pathway involved in the course of the diasease. Although it should be considered that the expression level of those genes in lymphocytes and in neurons are different from each other, they still differ on lymphocytes between AD patients and healthy groups, which bring us new information about it. [Financial support: FAPESP (Proc. 06/01947-8), CNPq, CAPES, RBC].

Multilocus gene-gene interaction analysis of osteoporosis in Chinese population. *X. Hong[1], H. Tsai[1], H. Dong[3], Z. Li[3], X. Liu[3], X. Xu[2], X. Wang[1]* 1) Mary Ann and J. Milburn Smith Child Health Research Program, Childrens Memorial Hospital and Childrens Memorial Research Center, Chicago, IL; 2) Center for Population Genetics, School of Public Health, University of Illinois at Chicago, Chicago, IL; 3) Institute of Biomedicine, Anhui Medical University, Hefei, Anhui, China.

Abstract: A number of osteoporosis candidate genes have been reported previously, but with inconsistent results. This study aimed to validate associations of eight reported genes with osteoporosis in a population-based Chinese cohort and to examine potential gene-gene interactions. A total of 1190 extreme low femoral neck bone mass density (fnBMD) cases and 1192 extreme high fnBMD controls were selected. We tested associations of 16 single nucleotide polymorphisms (SNP) in the 8 genes with extreme low fnBMD and osteoporosis using multiple logistic regression, with adjustment of important covariates. We also tested gene-gene interactions using multifactor-dimensionality reduction and conditional logistic regression. SNP rs1800872 in the IL10 gene and SNP rs1126667 in the ALOX12 gene were associated with an increased risk of extreme low fnBMD and osteoporosis in males only. SNP rs3778082 in the ESR1 gene was associated with osteoporosis in postmenopausal females only. However, none of those remained statistically significant after correcting for multiple testing. Importantly, we identified and validated a two-locus gene-gene interaction between rs12594287 in the CYP19A1 gene and rs2234693 in the ESR1 gene in males. Males with the AA/AG genotype in SNP rs12594187 in the CYP19A1 gene and CC/CT genotype in SNP rs2234693 in the ESR1 gene had a significantly lower risk of extreme low fnBMD (OR=0.47, 95%CI=0.32-0.68, p=0.00005) and osteoporosis (OR=0.25, 95%CI=0.13-0.51, p=0.0001). No gene-gene interaction was found in females. In conclusion: our results demonstrated gender specific genetic association and gene-gene interaction in osteoporosis.

VEGF 936C>T is predictive of retinopathy of prematurity in Japanese infants with gestational age of 30 weeks or less. *M. Yagi[1,2], M. Yamamori[3], I. Morioka[2], N. Yokoyama[2], N. Okamura[4], T. Nakamura[1], T. Sakaeda[5], M. Matsuo[2]* 1) Clin Evaluation of Pharmacotherapy, Kobe Univ Graduate Sch Med, Kobe, Japan; 2) Pediatrics, Kobe Univ Graduate Sch Med, Kobe, Japan; 3) Hospital Pharmacy, Kobe Univ Hospital, Kobe, Japan; 4) Clinical Pharmacy, Sch of Pharmaceutical Science, Mukogawa Womens Univ, Hyogo, Japan; 5) Frontier Education Center, Graduate Sch Pharmaceutical Sciences, Kyoto Univ, Kyoto, Japan.

Background: Retinopathy of prematurity (ROP) is a major problem among premature infants. ROP is characterized by abnormal neovascularization of the retina. It is known that vascular endothelial growth factor (VEGF), a major mediator of vascular permeability and angiogenesis, plays an important role in the pathogenesis of ROP. **Purpose:** To assess the association of ROP with *VEGF* genetic polymorphisms and clinical (maternal, perinatal, neonatal) parameters in the Japanese population. **Methods:** Sixty-seven infants with a gestational age of 30 weeks or less were enrolled and divided into two groups: threshold ROP group included patients who progressed in stage 3 and were treated (n=30); non-threshold ROP group included patients without ROP or with ROP stage 1or 2 (n=37). Genomic DNA was extracted from buccal mucosa or umbilical cord. Six polymorphisms in the *VEGF* gene were evaluated. **Results:** Genotypes distribution of *VEGF* 936C>T, but not -1498T>C, -1154G>A, -634C>G, -7C>T, or 1612G>A were significantly different between threshold and non-threshold ROP groups. Eleven of the tested clinical parameters were also significantly different between the two groups in the univariate analysis. Logistic regression analysis with adjustment for gestational age and birth-weight showed that the heterozygous or homozygous for the T-alleles of *VEGF* 936C>T (OR:4.41; 95%CI:1.05-18.61; P=0.043) and chronic lung disease (CLD) (OR:7.37; 95%CI:1.20-45.47; P=0.031) were independent risk factors of threshold ROP. **Conclusions:** This study showed that *VEGF* 936C>T and CLD as independent risk factors in the development of threshold ROP. *VEGF* 936C>T may be a predictor of ROP in Japanese infants with gestational age of 30 weeks or less.

A synonymous CT substitution in exon 15 of *UBE1* causes reduced expression in patients and carrier females from XL-SMA families. *A. Meindl[1], J. Ramser[1], C. Lenski[1], M. E. Ahearn[2], K. Yariz[2], M. von Rhein[3], B. Wirth[4], L. L. Baumbach[2]* 1) Dept OB/GYN, Klinikum Rechts der Isar, Munich, Germany; 2) University of Miami, Miami, FL, USA; 3) University Children's Hospital Mainz, Mainz, Germany; 4) Insititute of Human Genetics, University of Cologne, Cologne, Germany.

Recently, we have associated two missense mutations and one synonymous CT substitution (c.1731CT, p.Asn577Asn), all located in exon 15 of the *UBE1* gene, with X-linked infantile spinal-muscular atrophy (XL-SMA; MIM301830) (Ramser et al; AJHG 2008). XL-SMA is an X-linked motor neuron disorder that presents with the clinical features of hypotonia, areflexia and multiple congenital contractures associated with anterior horn cell loss and infantile death. *UBE1* codes for Ubiquitin-Activating Enzyme E1 that catalyzes the first step in the ubiquitin-proteasome system (UPS) which is responsible for intracellular degradation of proteins. While the consequences of the two missense mutations for the ubiquitin-proteasome pathway and the SMN complex stability are described in another abstract (Baumbach et al., this meeting), this abstract focuses on the investigation of the pathomechanism of the synonymous CT substitution. In a first step we have performed expression analysis applying quantitative RT-PCR in white blood cells of a still living XL-SMA patient displaying the synonymous substitution and revealed a significant reduction of *UBE1*-expression to one fifth as compared to three male healthy controls. Further expression studies on RNA from white blood cells of eight carrier females of three unrelated XL-SMA families carrying the synonymous substitution revealed a 50% reduction of the *UBE1* expression compared to eight female healthy controls. Since it has been shown that *UBE1* escapes X-inactivation, these results confirm that the synonymous CT substitution, which was shown to be absent in 7914 control X-chromosomes, leads to a significant reduction of *UBE1*-mRNA expression. Whether this reduction is caused by a possible modification of an exonic splice modulator element or by the alteration of the methylation pattern of a putative regulatory element present in exon 15 of *UBE1* is currently under investigation.

Recombination hotspots and haplotype blocks in three isolated Chinese Muslim communities. *M. S. Song[1], W. Wang[1,2], X. M. Lu[3], Y. X. Wang[1]* 1) Department of Epidemiology & Health Statistics, Capital Medical University, Beijing, China; 2) College of Life Sciences, Graduate University of Chinese Academy of Sciences, Beijing, China; 3) Medical Research Center, 1st Teaching Hospital, Xinjiang Medical University, Urumqi, Xinjiang Uygur Autonomous Region, China.

Detection of the patterns of recombination hotspots and haplotype blocks in the human genome is important both for understanding the nature of recombination and for applications such as association studies. The Chinese Muslim minorities are specific resources for such genetic analysis due to their unique culture and marriage practices. The study on the patterns of recombination hotspots and haplotype blocks in the isolated Chinese Muslim communities should aid in exploring the genetic resources of China. Three isolated Muslim communities, i.e. Salar, Boan and Dongxiang, which were resident in Jishishan Minority Autonomous County, Gansu province, northwest China, with a custom of endogamy and preferential consanguineous marriage, were taken as subjects of this study. The recombination hotspots within the human PGM1 gene were estimated by the analysis of SNP genotyping data using a population genetic approach (i.e. the HotspotFisher method). The analysis of haplotype structure of PGM1 was performed by a Haploview version 3.32 software package. The presence of both community-specific and common recombination hotspots with variable recombination rates and Log-likelihood ratio (LLR) were observed among the Salar, Boan and Dongxiang Communities. Different numbers of common haplotype blocks were detected in the three communities, i.e. nine in Salar, six in Baoan and eight in Dongxiang, respectively. The spatial arrangement of haplotype blocks within PGM1 of the three samples was mapped preferentially to the regions where LD collapses, corresponding with the location of hotspots and demonstrating that hotspot is a major factor determining the pattern of haplotype blocks. Our study suggests that investigation of the recombination hotspot patterns of various population isolates, would highlight genetic background of population samples involved in the association study of complex traits.

Genome-wide transcription profiling identifies genes influencing susceptibility to lipotoxicity and type 2 diabetes (T2DM). *S. Das[1,2], W. Chu[1,2], A. Mondal[1,2], N. Sharma[1,2], S. Elbein[1,2]* 1) Internal Medicine/Endocrinology, University of Arkansas for Medical Sciences; 2) CAVHS, Little Rock, AR.

Elevation in plasma free fatty acid (FFA) levels has been implicated in the development of T2DM by the effects on insulin resistance in peripheral tissues and -cell dysfunction. Human transformed lymphocytes (TLs) respond to FFA with increased markers of ER stress, hence making this a viable system to examine genetic influences of lipotoxicity. We hypothesized that T2DM individuals were genetically more susceptible to lipotoxicity, and that polymorphisms responsible for T2DM susceptibility would alter the expression of genes induced by FFA treatment. We selected 4 sib pairs from Caucasians selected to be discordant for T2DM, and exposed TLs to 0.5 mM palmitate or control for 6 hours. Genome-wide transcription profiling was performed using an Agilent 44K array. We detected 22,268 transcripts in all cells under both conditions. Palmitate treated cells showed up and down regulation (1.5 fold) of 123 and 17 genes respectively. The most up-regulated genes included DDIT3, STC2, ATF3 and CEBP, whereas the most down-regulated genes included CXCR3, XCL2, CMKLR1 and FUT7. Expression of DDIT3 was significantly correlated ($r^2$0.5; p0.05) with 2148 genes, but most strongly with MLLT10 (r=0.98) and LRP8 (r=-0.98). Differential expression of 32 and 29 transcripts between T2DM and normal cells was observed under control and palmitate treated conditions, respectively, and included significant differences in ARHGAP22, IFNG, IL18BP, PPAP2C, TNFSF11, CHDH, SLC12A7 and SLC6A6. Genes involved in ER stress response showed marked induction with palmitate. We thus examined ER stress genes in the same TLs under chronic stress condition (0.1mM palmitate for 2 passages followed by 0.5mM for 6hr). Neither T2DM nor normal TLs showed any evidence of adaptation to ER stress induced by palmitate, in contrast to HepG2 treated similarly. Our results indicate that genes involved in FFA metabolism and homeostasis differ between T2DM and nondiabetic siblings, and should be prioritized for analysis as functional candidate genes for T2DM.

Autosomal recessive transmission of male infertility due to Aurora Kinase C mutations is common in North Africa. *P. F. Ray[1,2], S. Hennebicq[1,2,3], R. Zouari[4], F. Vialard[5], R. Harbuz[1,2], K. Dieterich[1,2], H. Bellayou[6], I. Koscinski[7], M. R. Guichaoua[8], A. Zoghmar[9], M. Noruzinia[10], A. Sefiani[11], J. Lunardi[1,2]* 1) Genetique et Procreation, CHU Grenoble, France; 2) Faculté de Médecine-Pharmacie, Université Joseph Fourrier, France; 3) Inserm U823, Institut Albert Bonniot, Grenoble, France; 4) Centre de FIV les jasmins, Tunis, Tunisia; 5) Laboratoire de Cytogénétique, CHI Poissy-Saint Germain, France; 6) Laboratoire de Génétique. Faculté de Médecine et Pharmacie. Casablanca, Maroc; 7) CECOS d'Alsace, CMCO, Schiltigheim, France; 8) Biologie de la reproduction, Hôpital de la Conception, Marseille, France; 9) Médecine de la Reproduction, Cité Boussouf, Constantine, Algérie; 10) Sarem hospital, Ekbatan, Tehrran, Iran; 11) Génétique médicale, Institut National d'Hygiène, Rabat, Maroc.

Patients with primary infertility caused by 100% teratozoospermia with a majority of large-headed spermatozoa with up to four flagella were described 30 years ago (OMIM 24306). Several FISH studies demonstrated that all spermatozoa had gross chromosomal abnormalities, most of them being polyploid. We performed a genome-wide microsatellite scan and could localise a candidate region and identify the same homozygous mutation (c.144delC) in the Aurora Kinase C gene of all the tested men. All the patients had an identical haplotype indicating a founders effect(1). We have now genotyped a total of 37 patients with a typical large headed phenotype. All were mutated: thirty one were homozygous for c.144delC and we identified three novel mutations in 6 patients. No mutations were identified in any of the 7 AURKC exons of 32 patients with lower rates of macrocephalic (<30%) and multiflagelar (<10%) spermatozoa. Genotypes obtained from individuals from the North African general population (n=500) indicate a carrier frequency of 1/70-100. Although rare, the c.144delC mutation is thus probably responsible for the infertility of thousands of Maghrebian men. To our knowledge, AURKC is the first gene in which a recurrent mutation has been shown to cause male infertility by impairing spermatogenesis. 1. Dieterich et al.(2007) Nat Genet 39, 661-665.

A GWAS identifies common variations in the BARD1 tumor suppressor gene predisposing to high-risk neuroblastoma. *M. Capasso[1,2], M. Devoto[1,3], C. Hou[1], S. Asgharzadeh[4], E. Attiyeh[1], Y. Mosse[1,3], J. Bradfield[1], R. Scott[5], S. Diskin[1], J. Jagannathan[1], J. Glessner[1], C. Kim[1], W. London[6], R. Seeger[4], S. Grant[1,3], H. Li[3], N. Rahman[5], H. Hakonarson[1,3], J. Maris[1,3]* 1) The Childrens Hospital of Philadelphia, Philadelphia, PA, USA; 2) University Federico II, CEINGE - Biotecnologie Avanzate, Naples, Italy; 3) University of Pennsylvania School of Medicine, Philadelphia, PA, USA; 4) University of Southern California, Los Angeles, CA, USA; 5) Institute of Cancer Research, Sutton, Surrey SM2 5NG, UK; 6) University of Florida Gainesville, FL, USA.

Neuroblastoma (NB) is characterized by a high degree of clinical heterogeneity. Over half of all patients present with "high-risk" disease features including metastases at diagnosis and survival probabilities <40%. We recently discovered a significant association of common 6p22 SNPs with NB (Maris et al, NEJM 2008). Because this signal was enriched in the high-risk population, we performed a second analysis restricted to the high-risk subset by comparing SNP allele frequencies in 397 cases and 2,043 controls of European descent genotyped with the Illumina HH550 array. In addition to the 6p22 signal, we observed genome-wide significant association with 6 common intronic SNPs of the BARD1 tumor suppressor gene at 2q35 (allelic OR=1.56-1.64, P<10-7). Analysis using HapMap data showed that 4 of the associated SNPs are in strong LD with known BARD1 non-synonymous SNPs (r2>0.5). Association with the 6 BARD1 SNPs was replicated in an independent group of 189 high-risk cases and 1178 controls (allelic OR=1.52-1.80, P<10-3). The two most significant SNPs (rs6435862, rs3768716) were further replicated in two additional groups of high-risk patients from the UK (86 cases and 782 controls) and the US-based Children's Cancer Group (96 cases and 162 controls) using real-time PCR. The total combined allelic ORs and P-values were 1.66 and 8x10-19 for rs6435862 and 1.88 and 9x10-27 for rs3768716. These findings demonstrate that apart from its reported role in breast cancer, BARD1 is a susceptibility gene for NB, suggesting that the molecular mechanisms underlying these disorders may share common biological pathways.

Penetrance of Familial Pulmonary Arterial Hypertension is modulated by the expression of wild-type *BMPR2* transcript levels. *R. Hamid[1], J. Cogan[1], l. Hedges[1], J. Phillips[1], J. Newman[2], J. Loyd[2]* 1) Dept Pediatrics, Div Med Gen, Vanderbilt Univ Sch Med, Nashville, TN; 2) Dept. Medicine, Vanderbilt Univ Sch Med, Nashville, TN.

Rationale: Familial pulmonary arterial hypertension (FPAH) is a progressive, fatal disease caused by mutations in the *bone morphogenetic protein receptor type 2 (BMPR2)* gene. FPAH is inherited as an autosomal dominant trait and shows incomplete penetrance in that many with BMPR2 mutations do not develop FPAH, suggesting a role for, as yet unidentified, modifier genes in disease penetrance. Objectives: We hypothesized that variable levels of expression of the wild type (WT) *BMPR2* allele could act as a modifier and influence penetrance of FPAH. Methods: WT *BMPR2* levels were determined by real-time PCR analysis in lymphoblastoid (LB) cell lines derived from normal controls and individuals with sporadic PAH or FPAH. The FPAH kindreds analyzed carried mutations that result in the activation of nonsense mediated decay (NMD) pathway, which leads to the degradation of the mutant RNA thus ensuring that only the WT *BMPR2* transcripts will be detected in the real-time assay. Results: Our data show that WT and mutant *BMPR2* levels can be reproducibly measured in patient derived LB cell lines and that unaffected mutation carrier derived LB cell lines have higher levels of WT *BMPR2* transcripts than FPAH patient derived LB cell lines ($p<0.005$). Conclusions: Our findings suggest that the levels of expression of WT *BMPR2* allele transcripts is important in the pathogenesis of FPAH caused by NMD+ mutations. Furthermore, our study illustrates a novel application of lymphoblastoid cell lines in the study of PAH, especially important because the affected site, i.e. lung is not available for unaffected mutation carriers.

SURF and TuRF: Computationally efficient algorithms for detecting epistasis in genome-wide association studies. *J. Kiralis, C. Greene, N. Penrod, J. Moore* Computational Genetics Laboratory, Deparment of Genetics, Dartmouth College, Lebanon, NH.

Genome-wide association studies (GWAS) are becoming the de facto standard in the genetic analysis of common complex human disease. Given the complexity and robustness of biological networks, such diseases are unlikely to be the result of single points of failure but instead the joint failure of two or more interacting components. The promise of GWAS is that many of these points of failure can be traced to informative single nucleotide polymorphisms (SNPs). Detecting interacting variants that lead to disease in the absence of single-gene effects is difficult however, and methods to characterize these interactions are of exponential time complexity. In order to successfully model interactions in genome-wide data a heuristic which can detect such interactions is needed. The Relief algorithm was developed in 1992 as a machine learning algorithm that uses a nearest neighbor approach to assign weights to independent variables in a dataset that reflect their importance with respect to a dependent variable. We have previously introduced the novel Tuned ReliefF (TuRF) algorithm and have shown that it can effectively assign weights to SNPs in genome-wide association studies that reflect their role in epistatic interactions. Here, we introduce the Spatially Uniform ReliefF (SURF) algorithm that greatly improves the weights assigned to SNPs by assessing nearest neighbors within a given distance epsilon. To assess these novel algorithms we designed an artificial GWAS with cases and controls simulated using a wide variety of different epistasis models with heritabilities ranging from 0.025 to 0.4 and sample sizes ranging from 400 to 3200. We show here that the combined use of SURF and TuRF dramatically improves the power to highly rank functional interacting SNPs in the context of a GWAS. These results challenge the commonly held assumption that 10 nearest neighbors are sufficient for Relief algorithms. We anticipate SURF and TuRF will play a very important role in prioritizing SNPs for interactions analysis in GWAS of complex human diseases.

LONG-TERM (2-3 YEARS), OPEN-LABEL EXTENSION STUDY OF IDURSULFASE IN THE TREATMENT OF HUNTER SYNDROME. *J. Muenzer[1], E. Wraith[2], M. Beck[3], E. Wraith[4], P. Harmatz[5], C. M. Eng[6], A. Vellodi[7], R. Martin[8], U. Ramaswami[9], M. Calikoglu[1], S. Vijayaraghavan[2], A. C. Puga[4], B. Ulbrich[9], M. Shinawi[6], M. Cleary[7], S. Wendt[3]* 1) Univ North Carolina, Chapel Hill, NC; 2) Royal Manchester Children's Hospital, Manchester, UK; 3) Univ. of Mainz, Mainz, Germany; 4) Medical Genetics Service, HCPA/UFRGS, Brazil; 5) Children's Hospital, Oakland, CA; 6) Baylor College of Medicine, Houston, TX; 7) Great Ormond Street Hospital, London, UK; 8) St. Louis Children's Hospital, St. Louis, MO; 9) Cambridge Univ. Teaching Hospitals, Cambridge, UK.

Hunter syndrome (MPS II) is caused by a deficiency in iduronate-2-sulfatase. In the pivotal 1-year, double-blind, placebo-controlled clinical trial of enzyme replacement therapy with idursulfase (Elaprase, Shire HGT, Cambridge, MA), weekly dosing (0.5 mg/kg) significantly improved the primary endpoint (a composite comprising sum of the ranks of changes in percent predicted forced vital capacity (%FVC) and distance walked in 6 minutes (6MWT) compared to placebo). All patients who completed the double-blind study (n=94) enrolled in the open-label extension study and were treated with idursulfase at 0.5 mg/kg weekly for 2 years. The primary objective of this study was to collect long-term safety and clinical efficacy data from MPS II patients treated with idursulfase. All evaluations made during the double-blind study, including absolute and % predicted FVC, 6MWT, and organ size, were monitored throughout the open-label extension study. Safety was assessed continuously during the study by monitoring treatment emergent adverse events and by periodic determination of anti-idursulfase antibodies in blood samples. In an interim 1-yr analysis, patients in the original placebo group who transitioned to weekly idursulfase therapy (n=28) demonstrated an increase in absolute FVC (+6.8%, P=.005) and decreases in liver (-23%, P<.0001) and spleen volume (-17%, P=.0007) and urine GAG excretion (-66%, P<.0001). In these patients, no new safety issues emerged, and these results continued to support a positive risk-benefit ratio of weekly idursulfase use. The 2-year safety and efficacy results will be presented.

Molecular Characterization and Postmortem Review of Holoprosencephaly (HPE) Cases. *F. Lacbawan[1,2], A. Igbokwe[2], D. Pineda[1], M. Muenke[1]* 1) Med Gen Branch, NHGRI/NIH, Bethesda, MD; 2) SUNY Downstate Medical Center, Brooklyn NY.

HPE is the most common structural forebrain malformation in humans with genetic and environmental causes. Besides chromosomal abnormalities & submicroscopic *dels*, recurring mutations were found in *ZIC2, SHH, SIX3, TGIF1, GLI2*, & *FOXH1*. In our studies, there is variable expressivity and reduced penetrance in SIX3 mutation(+) cases and gonodal mosaicism in ZIC2 mutation(+) cases. A multiple hit hypothesis that predicts that several genetic &/or environmental insults are required to produce HPE in humans was proposed. In our continued efforts to characterize the clinical spectrum, we reviewed 48 cases with postmortem reports. DNA samples were screened for mutations in *ZIC2, SHH, SIX3* and *TGIF* by PCR of exons followed by sequencing. The age ranged from GA 15 wks to 8 yrs & F:M ratio was 2:1. 21 were diagnosed on prenatal USS. Maternal history was significant in only 3 (alcohol, smoking, diabetes & cocaine). 17(35%) had more than 1 clinically affected family member with known karyotype (KT) abnormalities in 4. There were also 4 of 31 sporadic cases with abnormal KT. In 1/2 of the cases with normal KT, we found 2 SHH mut(+) and 1 ZIC2 mut(+). Most cases reviewed were severe forms with 44% alobar, 25% semilobar and 13% lobar. Common craniofacial features were microcephaly, proboscis, olfactory bulb/tract agenesis, hypotelorism, CL/P, ear anomalies and cyclopia/anophthalmia. Other brain findings include corpus callosum a/dysgenesis(23%), cortical/cerebellar malformations(17%), hydrocephalus(15%) and migration defects(6%). 27% also had significant cardiac defects including ASD, VSD, PFO, and hypoplastic left heart. Associated findings included limb anomalies(27%) and GU anomalies like renal, uterine, anterior perineal anomalies and hypospadias(27%). Our review highlights the need for clinicians and pathologists to know the broad spectrum of anatomic features of HPE so that appropriate clinical diagnostic work-up like radiology and use of the algorithm of molecular and chromosomal studies are optimized. A better understanding of HPE pathogenesis will translate to a more comprehensive clinical management of patients and proper counseling of families.

Hypomorphic Mutations in Meckelin (MKS3/TMEM67) Cause Nephronophthisis with Liver Fibrosis. *E. Otto[1], K. Tory[2], M. Attanasio[1], Y. Paruchuri[1], E. Wise[1], B. Utsch[1], M. Wolf[1], C. Becker[3], G. Nuernberg[3], P. Nuernberg[3], A. Nayir[4], S. Saunier[2], C. Antignac[2], F. Hildebrandt[1]* 1) Dept Ped, Univ Michigan, Ann Arbor, MI; 2) Inserm, U574, Univ Paris Descartes, Paris, France; 3) Center for Genomics, Univ Cologne, Germany; 4) Dept Ped Nephrology, Univ Istanbul, Turkey.

Nephronophthisis (NPHP), a rare recessive cystic kidney disease, is the most frequent genetic cause of chronic renal failure in children and young adults. Mutations in 9 genes (*NPHP1-9*) have been identified, accounting for approximately 30% of all cases. NPHP can be associated with retinal degeneration (Senior-Loken syndrome), brainstem and cerebellar anomalies (Joubert syndrome), or liver fibrosis. Its molecular basis relates to functional defects of primary cilia. To identify a causative gene for the subset of patients with NPHP and associated liver fibrosis, we performed a genome-wide linkage search in a family with 5^{th} degree consanguinity and 3 affected children using homozygosity mapping and 50K Affimetrix SNP microarrays. We obtained a significant maximum parametric lod score of Z_{max} =3.72 on chromosome 8q22 and identified a homozygous missense mutation in the gene *MKS3/TMEM67*. When examining a world wide cohort of 62 independent patients with NPHP and associated liver fibrosis we identified altogether 4 novel mutations (p.W290L, p.C615R, p.G821S, and p.G821R) in 5 of them. Mutations of *MKS3/TMEM67*, found recently in Meckel-Gruber syndrome (MKS) type 3 are in 71% truncating mutations. In contrast, the mutations detected here in patients with NPHP and associated liver fibrosis were exclusively missense mutations. This suggests that they may represent hypomorphic alleles, leading to a milder phenotype compared with the more severe MKS phenotype. Additionally, mutation analysis for *MKS3/TMEM67* in 120 patients with Joubert syndrome (JBTS) with associated kidney disease yielded 7 different (6 novel) mutations in 5 patients, 4 of which presented with congenital liver fibrosis. In 105 patients with NPHP without liver or brain involvement no mutations were found. We conclude that hypomorphic MKS3 mutations can cause NPHP (type 11) with liver fibrosis. Thus, NPHP11, MKS3 and JBTS6 are allelic disorders.

The effects of functional DNA elements in cognition-related genes on delay discounting and adolescent cigarette smoking behaviors. *P. J. Weaver[1], T. R. Simmons[1], T. Andrews-Bryant[1], B. Reynolds[2, 3], C. W. Bartlett[1, 3]* 1) Battelle Center for Mathematical Medicine, The Research Institute at Nationwide Children's Hospital, Columbus, OH; 2) Center for Biobehavioral Health, The Research Institute at Nationwide Children's Hospital, Columbus, OH; 3) Department of Pediatrics, College of Medicine, The Ohio State University, Columbus, OH.

Given that most adult smokers begin smoking during adolescence, understanding smoking behaviors among adolescents is crucial to the development of strong public health policies. Approximately 4,000 adolescents between the ages of 12 and 17 initiate cigarette smoking every day, and of those an estimated 1,140 become daily smokers. Identification of genetic factors related to smoking has significant implications, though genetic variants directly associated with smoking are limited. Using alternative, smoking-related phenotypes such as impulsivity, the present study examines the relationship between these phenotypes through a unique cognitive panel titled CP1. Adolescents who were designated as smokers, non-smokers, or as having tried smoking were rated for impulsivity using measures of delay discounting. DNA was then collected and analyzed using CP1, which is comprised of 29 SNPs, all of which have shown evidence of regulatory effects on gene expression in genes associated with cognition. This panel utilizes 29 DNA primer sets in a single PCR, which is then followed by a ligase detection reaction (LDR). The LDR is performed on the amplicons to interrogate the specific alleles; each coupled to a single fluorescently dyed microsphere. Finally, we use the Luminex 200 to visualize all members of the panel and quantify the signal intensity of each individual allele. To validate the CP1 SNPs, we genotyped a series of 4 extended pedigrees from the anonymous, publicly available CEPH collection. This comparison allowed for error checking (via missegregation of alleles in the pedigrees) as a measure of which assays could be successfully multiplexed together. The goal of this study then, is to identify the main effects of these SNPs, thereby studying the genetic relationship with delay discounting and smoking related behaviors.

Additional high-risk melanoma susceptibility genes. *A. M. Goldstein, Melanoma Genetics Consortium (GenoMEL)*
Genetic Epidemiology Br, DCEG, National Cancer Inst, NIH, DHHS, Bethesda, MD.

CDKN2A/ARF and CDK4 are the high-risk melanoma susceptibility genes identified to date. CDKN2A/ARF encodes two distinct proteins translated, in alternate reading frames (ARF), from alternatively spliced transcripts. Although several recent linkage analyses and candidate gene searches have been conducted, no additional high-risk melanoma susceptibility genes have yet been identified. Using data from GenoMEL, we illustrate why finding additional high-risk melanoma susceptibility genes has been so challenging. GenoMEL created the largest familial melanoma data set yet assembled; it included 466 families with 3 melanoma patients from 17 GenoMEL centers from Europe, Australia, the Middle East, and North America. Germline CDKN2A mutations were detected in 38% of families. Mutations in CDK4 and ARF were much rarer and occurred at similar frequencies, 2-3%. No obvious melanoma or pigmentation-related clinical characteristics distinguished families with CDKN2A mutations from those with CDK4 or ARF mutations. The number of melanoma patients in a family was also strongly and directly associated with mutation detection and showed substantial variation by continent. Using the observed distribution of CDKN2A, CDK4, and ARF mutations by continent and number of melanoma patients per family (3, 4-5, 6), we estimated the number of additional high-risk melanoma susceptibility genes that would be expected in this study sample. Assuming additional high-risk susceptibility genes showed distributions similar to CDK4 and ARF we would expect about seven rare high-risk melanoma susceptibility genes to be detected in about 40 of the 276 mutation negative families. In contrast, a high-risk melanoma susceptibility gene similar to CDKN2A would result in approximately 89 mutation positive families. The results suggest that if additional high-risk melanoma susceptibility genes are similar to CDK4 and ARF, both in frequency and distribution of melanoma patients, traditional methods such as linkage analysis may be very challenging for finding these genes and other detection approaches will be needed.

Copy Number Analysis in Patients with Congenital Heart Disease and Multiple Congenital Anomalies. *P. Paluru[1], J. Garbarini[1], X. Gai[2], J. Glessner[3], J. Biegel[4], H. Hakonarson[3], P. S. White[2,5], T. Shaikh[4], E. Goldmuntz[1]* 1) Division of Cardiology, Children's Hosp Philadelphia, Philadelphia, PA; 2) Center for Bioinformatics, Children's Hosp Philadelphia, Philadelphia, PA; 3) Center for Applied Genomics, Children's Hosp Philadelphia, Philadelphia, PA; 4) Division of Human Genetics, Children's Hosp Philadelphia, Philadelphia, PA; 5) Division of Oncology, Children's Hosp Philadelphia, Philadelphia, PA.

Congenital heart defects (CHD) are among the most common birth defects and are the leading cause of birth defect-related deaths. About 25% of CHD occur in conjunction with other congenital defects and are often part of a specific malformation pattern or genetic syndrome. Large chromosomal alterations, submicroscopic deletions and single gene defects have been identified in a subset of genetic syndromes characterized in part by CHD. We hypothesized that subjects with CHD and additional dysmorphic features or congenital anomalies would be a likely cohort to harbor previously undetected copy number alterations (CNAs). We evaluated 58 subjects with a range of CHD and other dysmorphia and/or congenital anomalies, who did not have a recognized genetic syndrome or chromosomal abnormality. The cohort included patients with left sided lesions (n=16), conotruncal defects (n=20), septal defects (n=18), and other abnormalities (n=4). Subjects were assessed for CNAs using Affymetrix 100k SNP array technology and analytical approaches. We identified 13 unique CNAs including 9 deletions and 4 duplications, that were not present in the Database of Genomic Variants or in over 2,000 control subjects genotyped at our institution. Each of the 13 CNAs were confirmed by alternative methods. The CNAs range from 0.05-9.6Mb in size and are predicted to contain either single or many genes with variable functions. Eight of the CNAs were confirmed to be inherited; some represent unique regions of relative copy number gain or loss. These data may identify new disease loci and/or candidate genes that warrant further evaluation in similarly syndromic and non-syndromic cardiac cohorts. We present the first study of CNAs to focus specifically on subjects with congenital heart disease.

Revisiting sum statistics for detecting epistasis in genome-wide association studies. *C. Greene, J. Moore*
Computational Genetics Laboratory, Department of Genetics, Dartmouth College, Lebanon, NH.

Picking a group of SNPs that predict an individual's disease risk is a crucial step in genome-wide association studies (GWAS). However, this is not a trivial exercise when the SNPs in question are involved in nonlinear interactions. Sum statistic methods have been explored in conjunction with chi-square statistics to find a set of SNPs significantly associated with the clinical endpoint being studied. This is a promising approach because it considers sets of SNPs rather than individual SNPs. However, sums of chi-square statistics may fail to identify certain types of gene-gene interactions. Here we introduce a novel combination of sum statistics and ReliefF as a method for picking a significant set of SNPs from a GWAS. The Relief algorithm was developed in 1992 as a machine learning algorithm that uses a nearest neighbor approach to assign weights to independent variables in a dataset that reflect their importance with respect to a dependent variable. We have previously introduced the novel Tuned ReliefF (TuRF) algorithm and have shown that it can efficiently assign weights to SNPs in GWAS that reflect their role in epistatic interactions. Our goal was to compare the power of sum statistics using chi-square values with sum statistics using TuRF scores for detecting interacting SNPs in a GWAS. To accomplish this goal we designed a simulated GWAS with cases and controls simulated using a wide variety of different epistasis models with heritabilities ranging from 0.025 to 0.4 and sample sizes ranging from 400 to 3200. We show that sum statistics that use TuRF scores significantly outperform sum statistics using chi-square values across a wide range of different epistasis models ($P<0.05$). These results support the idea that sum statistics will be useful for detecting epistasis in GWAS when a powerful measure of SNP quality such as ReliefF is used.

Failure to replicate a genetic association may provide important clues about genetic architecture. *N. Penrod, C. Greene, J. Moore* Computational Genetics Laboratory, Department of Genetics, Dartmouth College, Lebanon, NH.

Replication has become the gold standard for assessing statistical results from genome-wide association studies. Unfortunately, a real result can fail to replicate for a number of reasons including inadequate sample size or variability in phenotype definitions across independent samples. We hypothesize that some statistically significant independent genetic effects may fail to replicate in a second dataset when the underlying genetic architecture is complex and the functional polymorphism interacts with one or more other functional polymorphisms. This hypothesis is based on the idea that a functional polymorphism with an interaction effect may fail to replicate a significant independent effect due to allele frequency differences between the detection sample and the replication sample. To test this hypothesis we designed a simulation study in which case-control status is determined by two interacting polymorphisms with heritabilities ranging from 0.025 to 0.4 and replication sample sizes ranging from 400 to 1600. Our results across this wide range of epistasis models with differing heritabilities and sample sizes show that the power to replicate a statistically significant independent effect of a polymorphism can drop from 1.0 to 0.05 with a change of allele frequency of less than 0.1 for the interacting partner. These results support our hypothesis and suggest that failure to replicate an independent genetic effect may provide some clues about an underlying genetic architecture that may include gene-gene interactions or epistasis. We recommend that polymorphisms that fail to replicate be checked for interactions with other polymorphisms, especially when there are allele frequency differences between the detection and replication samples for multiple polymorphisms.

Pericentrin molecular analysis in 21 Seckel/MOPDII patients. *M. Willems[1], D. Geneviève[1], G. Borck[1], G. Baujat[1], M. Gérard[2], D. Héron[3], B. Leheup[4], M. Le Merrer[1], A. Verloes[2], L. Colleaux[1], A. Munnich[1], V. Cormier-Daire[1]* 1) Dept of Genetics, INSERM U 781, Hôpital Necker, Paris, France; 2) Dept of Genetics, Hôpital Robert Debré, Paris, France; 3) Dept of Genetics, Hôpital de la Pitié-Salpêtrière, Paris, France; 4) Dept of Genetics, CHU Nancy, Hôpitaux de Brabois, Nancy, France.

Among the primordial dwarfisms, microcephalic osteodysplastic primordial dwarfism type II (MOPD II, MIM 210720) and Seckel syndrome (SCKL, MIM 210600), are both characterized by intrauterine growth retardation, severe proportionate short stature and microcephaly. MOPDII is distinct from SCKL by more severe growth retardation, radiological abnormalities and milder mental retardation. SCKL is associated with defective ATR-dependent DNA damage signalling, but only a single hypomorphic mutation of ATR (Sckl1, 3q22.1-q24) has been yet identified in this genetically heterogeneous condition. In 2008, mutations in the gene encoding pericentrin (PCNT) have been identified in 28 patients, including 3 SCKL (Griffith et al, Nat Genet) and 25 MOPDII (Rauch et al, Science). This gene encodes a centrosomal protein, with key functions, anchoring both structural and regulatory proteins. We performed the direct sequencing of PCNT in 21 cases, including 15 consanguineous families (12 SCKL and 3 MOPDII) and 6 sporadic cases (4 SCKL and 2 MOPD II). We identified nine distinct mutations in 4/16 SCKL and 5/5 MOPDII, namely four stop mutation in exons 11, 15, 28 and 34, three frameshift mutations in exons 16, 30 and 30, one splice site mutation in intron 18, and one missense mutation in exon 19 (c.3840G>C, p.Q1280H). This mutation was absent in 200 controls and was presumably associated with an abnormal splicing resulting in pericentrin loss-of-function. The clinical analysis of the 4 SCKL cases with PCNT mutations showed that they finally all presented minor skeletal changes and a severe growth retardation more suggestive of MOPDII. We therefore conclude that, despite variable clinical severity, MOPDII is a genetically homogeneous condition due to loss-of function of pericentrin.

Multifactor Dimensionality Reduction 2.0: Open Source Software for Genome-Wide Analysis of Epistasis. *P. Andrews, J. Moore* Computational Genetics Laboratory, Department of Genetics, Dartmouth College, Lebanon, NH.

Multifactor dimensionality reduction (MDR) was designed as a nonparametric and genetic model-free approach to identifying, characterizing and interpreting gene-gene interactions in genetic and epidemiologic studies of common human diseases. The kernel of the MDR algorithm uses constructive induction to combine two or more polymorphisms into a single predictor that captures interaction effects. This general approach has been validated in numerous simulation studies and has been applied to a wide-range of different human diseases including asthma and allergy, autoimmune diseases, cancer, cardiovascular diseases, diabetes and metabolic syndrome, Mendelian diseases, pharmacogenetics and psychiatric diseases, for example. We describe here version 2.0 of the open-source MDR software package that has been made freely available to the genetic epidemiology and bioinformatics communities since February of 2005. This new version includes an estimation of distribution algorithm (EDA) for carrying out a stochastic search for the optimal combination of interacting polymorphisms. The new EDA algorithm provides an alternative to exhaustive search that may not be computationally feasible when the number of polymorphisms is large as in a genome-wide association study (GWAS). The key feature of this new algorithm is the ability to use expert knowledge in the form of prior statistical evidence (e.g. LOD scores, ReliefF) or biological evidence (e.g. chromosomal location, KEGG pathway, Gene Ontology) to probabilistically select polymorphisms for consideration in an MDR model. Previous studies have shown that detecting interactions in the absence of statistically significant independent effects in GWAS is not computationally feasible without expert knowledge. We anticipate this new version of MDR will open the door to the routine modeling of nonlinear interactions when the number of measured polymorphisms is large.

The Database of Genomic Variants (DGV) - Annotating structural variation in the human genome. *L. Feuk[1, 2], B. Thiruvahindrapduram[1, 2], J. R. Macdonald[1, 2], J. Zhang[1, 2], S. W. Scherer[1, 2]* 1) Program in Genetics & Genome Biology, The Hospital for Sick Children, Toronto, ON, Canada; 2) The Centre for Applied Genomics, The Hospital for Sick Children, Toronto, ON, Canada.

The Database of Genomic Variants (DGV) is a comprehensive and curated catalogue of structural variation in the human genome (http://projects.tcag.ca/variation/). DGV facilitates the interpretation of structural variation data in relation to previously published work. The content is focused on variants described in control samples, thus providing a valuable resource for both clinical and research labs producing structural variation data using array or next-generation sequencing approaches. The current content of the database is based on results from 48 peer reviewed research articles, representing a total of 25,383 variants. These structural variation entries are composed of 15,466 copy number variants (CNVs; defined as gains and losses of DNA segments over 1kb in size), 9,735 InDels (insertions and deletions of 100bp-1kb in size) and 182 inversions. Merging of overlapping CNVs results in a dataset contains 5,083 non-redundant CNV regions. The data are represented in table format, genome browser format, and text files available for download. The genome browser, which is based on the widely used GMOD software (GBrowse), is ideal for viewing structural variation in relation to other genomic features, such as genes, clones and segmental duplications. The database has been designed to be easily navigated and suitable for all users, independent of bioinformatics experience. The data in DGV has been produced with different technologies and analysis methods, which creates challenges with presentation and comparison between datasets. Current work in progress includes separating BAC array data from higher resolution platforms, a new track of known deletion/duplication syndromes to facilitate interpretation, and adding a new section to the database with CNV information for the mouse genome. Here we present an overview of DGV along with future plans for its expanded content and improved presentation.

Genome-wide association scan for the five major dimensions of personality. *A. Terracciano[1], S. Sanna[2], M. Uda[2], B. Deiana[2], EP. Slagboom[4], DI. Boomsma[5], S. Villafuerte[6], M. Burmeister[6], AC. Janssens[7], CM. van Duijn[7], WM. Chen[3], D. Schlessinger[1], GR. Abecasis[3], PTJr. Costa[1]* 1) National Institute on Aging, NIH, Baltimore, MD; 2) Istituto di Neurogenetica e Neurofarmacologia, CNR, Cagliari, Italy; 3) Center for Statistical Genetics, Department of Biostatistics, University of Michigan, Ann Arbor, MI; 4) Leiden University Medical Centre, Molecular Epidemiology Section, Leiden, The Netherlands; 5) Department of Biological Psychology, VU university, Amsterdam, The Netherlands; 6) Molecular & Behavioral Neuroscience Institute, Department of Psychiatry and Human Genetics, University of Michigan, Ann Arbor, MI; 7) Erasmus University Medical Center Rotterdam, The Netherlands.

Personality traits are summarized by five broad dimensions with pervasive influences on major life outcomes, strong links to psychiatric disorders, and clear heritable components. To identify genetic variants associated with each of the five major dimensions of personality we performed a genome wide association (GWA) scan of 3,972 individuals from a genetically isolated population within Sardinia. Based on analyses of 362,129 single nucleotide polymorphisms (SNPs) we found several strong signals within or near genes previously implicated in psychiatric disorders. They include the association of Neuroticism with SNAP25 (P = 5 X 10-5), Extraversion with BDNF and two cadherin genes (CDH13 and CDH23; Ps 5 X 10-5), Openness with CNTNAP2 (P = 3 X 10-5), Agreeableness with CLOCK (P = 9 X 10-6), and Conscientiousness with DYRK1A (P = 3 X 10-5). Effect sizes were small (less than 1% of variance explained), and most failed to replicate in the follow-up independent samples (N up to 3903), though the association between Agreeableness and CLOCK was supported in two of three replication samples (overall P = 2 X 10-5). We infer that a large number of loci may influence personality traits and disorders, requiring larger sample sizes for the GWA approach to identify significant genetic variants.

Simultaneous scan of genome-wide association data allowing for epistasis prioritizes multiple interacting loci in type 2 diabetes. *J. T. Bell[1,2], N. J. Timpson[1,3], N. W. Rayner[1], A. P. Morris[1], E. Zeggini[1], M. I. McCarthy[1], UK Type 2 Diabetes Consortium* 1) Univ Oxford, UK; 2) Univ Chicago, USA; 3) MRC CAiTE, UK.

Large-scale genome-wide association (GWA) data allow for systematic scans including epistasis, but at present such analyses are computationally and methodologically challenging. We undertook interaction analyses within data from the Wellcome Trust Case Control Consortium (WTCCC) GWA scan containing 1924 type 2 diabetes (T2D) cases and 2938 controls, with the aim of prioritizing pairs of markers for follow-up study. We performed a two-dimensional genome-wide scan using a joint two-locus test of association including main and epistatic effects in a subset of 70,236 markers tagging common SNP variation at $r^2>0.2$ and MAF0.05. The markers were derived from the tagging of 393,242 genotyped SNPs from the WTCCC Affymetrix chip data which passed stringent quality control. We examined 2.34×10^9 comparisons involving pairs of inter-chromosomal SNPs, and found that 79 pairs showed evidence for two-locus association at a Bonferroni-corrected P-value 0.05 (uncorrected P-value 2.14×10^{-11}). All of the 79 pairs involved loci that showed evidence for joint effects on T2D in cooperation with *TCF7L2*, which has strong single-locus effects. The pair-wise results included confirmed variants in *FTO* and *CDKAL1*, which exhibited significant main-effects and no evidence for epistasis with *TCF7L2*. However, the majority (>85%) of the 79 tag-SNPs did not have compelling single-locus evidence for association (P-value 1×10^{-4}). Epistatic analyses revealed 5 pairs of SNPs with significant additive-by-additive epistasis both in the case/control analyses and in analyses restricted to the T2D cases alone. The 5 loci included tag-SNPs located near *SLC9A9* (rs17635531), and regions on chromosomes 4 (rs1450140), 6 (rs1122637; rs1935683) and 11 (rs12803308). Our findings allow us to prioritize pairs of regions for follow up study in the extended data set. These analyses reveal that systematic genome-wide scans including epistasis are computationally feasible in GWA data, but the appropriate correction for multiple testing will affect the power to detect interactions and requires further study.

Extensions of Conditional Likelihood Bias Adjustment for Disease Association Risk Estimates in Whole-Genome Scans. *F. Zou, A. Ghosh, F. A. Wright* Department of Biostatistics, University of North Carolina, Chapel Hill, NC.

It is widely recognized that genome-wide association studies suffer from inflation of the risk estimates for genetic variants (usually SNPs) identified as significant in the genome scan, a so called "winner's curse". To handle such significance bias, we have developed an approximate conditional likelihood approach that can be applied using odds ratio estimates provided by standard statistical software. We have also developed a principled method to construct confidence intervals for the genetic effect that acknowledges the conditioning on statistical significance. We discuss extensions to the situation where risk estimation is performed for multiple correlated phenotypes in the genome scan. Our approach is widely applicable, is far easier to implement than competing approaches. The results have considerable importance for the proper design of follow-up studies and risk characterization.

Combined Intracerebroventricular/Intraperitoneal Enzyme Replacement Therapy Improves Survival and Reduces Brain Psychosine in a Mouse Model of Krabbe Disease. *L. Sturk[1], W. C. Lee[2], J. Pan[1], A. Herdt[2], G. S. Robinson[1], M. Concino[1], M. Heartlein[1], A. O. Tzianabos[1], C. B. Eckman[2]* 1) Shire Human Genetic Therapies, Cambridge, MA; 2) Department of Neuroscience, Mayo Clinic College of Medicine, Jacksonville, FL.

Globoid cell Leukodystrophy (GLD) or Krabbe disease is an autosomal recessive lysosomal storage disorder that occurs at an incidence of 1:100,000 births. A progressive peripheral (PNS) and central nervous system (CNS) disorder, GLD is the result of genetic mutations causing a deficiency in the enzyme galactocerebrosidase (GALC). We have used the twitcher mouse model to examine the effects of enzyme replacement therapy (ERT) on key endpoints including survival, psychosine levels, and sciatic nerve myelination status. Initial studies suggest that weekly intraperitoneal (i.p.) delivery of murine GALC (mGALC) extends survival and improves myelination of the sciatic nerve. A single intracerebroventricular (ICV) direct injection improved survival (51 1.6 days) compared to vehicle-treated animals (36 0.4 days) in a dose-responsive manner (40 g ICV = 42 1.7 days; 120 g ICV = 49 1.3 days). Brain psychosine levels were reduced following a single ICV injection at both low (39% reduction) and high (63% reduction) dose levels, to a greater degree than observed after i.p. administration. However, the sciatic nerve myelination was unimproved in ICV-treated mice as the vast majority of enzyme may be trapped within brain tissue after ICV administration. Twitcher mice treated with weekly i.p. mGALC combined with a single ICV injection at post-natal day (PND) 20 lived significantly longer (51 1.6 days) with normalized sciatic nerve myelination and dramatically reduced brain psychosine levels. Data from these studies suggest that optimal efficacy in the twitcher mouse model of Krabbe disease may require therapy that targets the PNS and CNS.

BiDil: Media Repersentations of an Emerging "Pharmacogenomic" Medication. *S. Harry* Health Law Inst, Edmonton, AB, Canada.

The print media occupies a pivotal role in informing the lay public of advances in medical treatments. Indeed, studies have shown the type of information disseminated or withheld by medical reporters, influences the attitudes of laypersons toward emerging pharmacogenetic technologies. As such we were particularly intrigued by the medias treatment of BiDil, a combination heart failure medication patented and characterized as the first race-based drug specifically for African Americans. The A-HeFT clinical trials on which the drug gained Food and Drug Administration (FDA) approval have been a source of controversy in the scientific community. The debate played out in a number of scientific journals between supporters of the drug who hail it as the first step towards decreasing health disparities experienced by African Americans as well as the first step towards pharmacogenomic medicine. Critics, meanwhile vocally oppose the drug, calling into question the methodology of the clinical trials, and interpretation of the results. In this charged situation, it is essential to understand the position of the print media and how they portrayed the BiDil story thereby shaping the perceptions of the potential consumers of this medication. This study examines popular representations of BiDil in major North America newspapers, using a coding frame successfully implemented in other media studies. The results indicate the media representation of BiDil was more complex than we first suspected. In some respects it supported the findings of previous studies on pharmaceuticals in the media in that the majority of articles emphasized the benefits of the drug while virtually excluding information on the side effects and contra-indications. Further, there was more emphasis on the strengths of the clinical trials than weaknesses. However, in other respects, the articles diverged from our expectations by portraying BiDil in a critical light by placing heavy emphasis on the potential problems with race-based pharmaceuticals such as the potential for racism. :.

Delineation of the breakpoints of pure duplication 3q due to a de novo duplication event using SOMA. *O. Nahum[1], B. Levy[1], A. Shanske[2]* 1) Department of Pathology, Columbia University. New York, NY; 2) Center for Craniofacial Disorders, Children's Hosp at Montefiore, Albert Einstein Coll of Med, Bronx, NY.

Partial duplication of distal 3q is a well-described condition of multiple congenital anomalies that resemble the Cornelia de Lange syndrome (CDLS). Most cases of dup(3q) syndrome result from an unbalanced translocation or inversion. Pure duplication of 3q is very rare and only a handful of cases have been reported. EC was a FT 3160g baby born to a 26 yr old primip by NSVD. He remained in the NICU for 10 days because of feeding difficulties. He was admitted to our hospital at 5 wks because of apnea and bradycardia. His physical examination at 4½ months revealed a stigmatized youngster. His weight was 5.42 kg, length 58.5 cm and HC 39 cm all below the 2%. He had bushy eyebrows, long eyelashes, synophrys and a nevus flammeus of the forehead. The pinna were crumpled, the nasal bridge was depressed, the philtrum long and the jaw micrognathic. He had bilateral syndactyly of toes 2 and 3 and a remnant of a right-handed post-axial polydactyly and bilateral 5th digit clinobrachydactyly. His psychomotor development was severely delayed and he was hypotonic. A sleep study revealed central apnea. A CT scan revealed craniosynostosis, absence of the corpus callosum & decreased white matter. Cytogenetic analysis revealed 46, XY .ish dup(3)(q21q29)(wcp3+,D3S4560+). Additional SOMA studies using the Affymetrix GenomeWide Human SNP Array 6.0 indicated the duplication to be 61.07 Mb with proximal and distal breakpoints at 3q22.2 & 3q29 respectively. The etiology of CDLS is heterogenous and about half the cases are due to mutations in the NIPBL gene on 5p13. Other causes include mutation in the X-linked SMC1L1 gene and mutation in the SMC3 gene on 10q25. The significant phenotypic overlap of CDLS with the duplication 3q syndrome indicates that an additional gene causing CDLS is located on distal 3q and recent studies have narrowed the region to 3q26.3. Our case confirms the association of duplication 3q with the CDLS and the precise delineation of the duplicated region by SOMA will help us to identify genes that contribute to the clinical features observed in the CDLS and the dup 3q syndrome.

Interaction between hemochromatosis and transferrin receptor genes with breast cancer in Sao Miguel population (Azores, Portugal). *P. R. Pacheco[1,2], M. J. Brilhante[1], T. Eloi[3], C. C. Branco[1,2], R. Cabral[1,2], V. Santos[3], V. Carneiro[4], L. Mota-Vieira[1,2]* 1) Molec Genetics, Pathol Unit, Hosp Divino Espirito Santo EPE, Azores, Portugal; 2) Instituto Gulbenkian de Ciência, Oeiras, Portugal; 3) Chirurgic Department, Hosp Divino Espirito Santo EPE, Azores, Portugal; 4) Anatomic Pathology Department, Hosp Divino Espirito Santo EPE, Azores, Portugal.

Two different *HFE* mutations (C282Y and H63D) have been found to increase cellular iron uptake and, due to the pro-oxidant properties of iron, altered iron metabolism in hemochromatosis patients might be a potential risk for breast cancer (BC). Some studies concluded that BC risk increase is also dependent on *HFE*-interacting genes, such as the transferrin receptor (*TFR*) and the combination between *HFE-TFR* genotypes. To assess if *HFE* mutations, *TFR*-S142G polymorphism and *HFE-TFR* genotypes are related to BC risk, we compared C282Y, H63D and S142G frequencies in 86 BC women and in 183 gender/age matched healthy controls. Samples were obtained after written informed consent. The C282Y allele frequency in the BC group was 4.07%, higher than in control group, 3.28%; while H63D mutation showed a similar frequency in BC, 21.51%, and controls, 21.04%. Although both groups were stratified according to menopausal status, odds ratio (OR) analysis for cancer risk associated with *HFE* mutations was not statistically significant. Regarding S142G polymorphism, the frequency of S142S, S142G and G142G genotypes were equivalent in both groups. In order to extend the search for a supposed BC susceptibility for *HFE-TFR* genotypes, we analysed BC and controls according to compound genotypes. Again, OR for all *HFE-TFR* genotype combinations revealed no increased risk for BC. In conclusion, the results suggest that *HFE* mutations are not associated with an increased risk for BC. *TFR* polymorphism was not an independent risk factor and did not modify the disease risk. Furthermore, variants of the *HFE-TFR* have, apparently, no direct effect on the incidence of breast cancer in the Azorean female population. (paularpacheco@hdes.pt). Azorean Government funded.

Analysis of DNA methylation in human placentae using the Illumina GoldenGate Methylation Panel I. *W. P. Robinson, L. Avila, M. Peñaherrera, P. von Dadelszen, M. S. Kobor* Univ British Columbia, Vancouver, BC, Canada.

Repetitive elements and X-linked gene promoters have been reported to have less DNA methylation in human placenta as compared to embryonic tissues, and instability of epigenetic programming has been suggested to contribute to placental insufficiency. However, patterns of DNA methylation have not been well-studied in an unbiased and genome-wide fashion. To investigate this, the Illumina GoldenGate Methylation Cancer Panel I (1,505 CpG loci from 807 genes) was used to interogate DNA extracted from placental villi (N=20) and whole blood (N=7). The distribution of beta values (% methylation) was similar, with the majority (59% in blood, 53% in placenta) of CpG-island associated CpGs (N=1044) being hypomethylated (beta value <10%), while most non-island associated CpGs (N=461) were highly methylated (beta value>75%) in both tissues. While no difference was observed in sample methylation averaged across all non-CpG island sites, placental samples had a higher mean methylation for CpG sites located in islands (22.2% vs. 17.7% in blood, P<0.0001). Variability was greater in the placenta, with a mean sd across autosomal sites of 0.079 compared to 0.052 for blood. Hierarchechal clustering showed that the epigenetic profile of placenta was distinct from blood: intra-placental correlations (based on 5 placentae with 2 villous samples each) ranged from 0.94-0.98, inter-placental sample correlations ranged from 0.91-0.96 but the average blood-placenta correlation was only 0.71. A number of sites showed completely discordant (hyper/hypo) patterns of methylation in placenta compared to blood. DNA methylation profiles from placentas associated with IUGR (N=10), preeclampsia (N=14), and CPM16 (N=6) were also analyzed with multiple significant differences compared to controls identified. These findings are being confirmed and extended using a locus specific approach (pyrosequencing). Identification of epigenetic differences between complicated and uncomplicated pregnancies are providing insight into the underlying placental abnormalities and may provide tools for non-invasive prenatal screens for the future.

New tool (mtPHYL) proposed for phylogenetic analysis of human complete mitochondrial genomes. *N. Eltsov, N. Volodko, E. Starikovskaya, R. Sukernik* Laboratory of Human Molecular Genetics, Institute of Cytology and Genetics SB RAS, Novosibirsk, Russian Federation.

The mitochondrial genome is widely used system to study evolutionary history of our species. With the advent of human population genomics (Hedges, 2000, Nature 408:652-653) and rapid accumulation of complete mtDNA sequences available through GenBank, it has become important to expedite the estimation process of increasingly accumulating mtDNA data sets. The central idea behind maximum parsimony is to fit character data to a semi-labeled tree so as to minimize the number of reverse and convergent transitions. The computational effort of standard methods grows exponentially so they are practically inapplicable with large data sets. To solve this problem we have elaborated a novel multistage algorithm. Preliminary analysis reduces an amount of the enumerations and, hence, allowing to reconstruct phylogenetic tree of large datasets at a minimum time-period. The algorithm which we created was implemented in the mtPHYL. This program reconstructs the phylogenetic trees and calculates the respective ages for the clusters within the tree. It can be used to glean a bulk of entire mitochondrial sequences from GenBank database instantly. In addition, it automatically categorizes the mutations and identifies affected genes along with their conservation indices and amino acid replacements. Our software may be easily modified to analyze any non-recombining DNA regions. mtPHYL is available from authors upon request (eltsovnp@bionet.nsc.ru) and at www.bionet.nsc.ru/labs/mtgenome/programs.html.

A *de novo* constitutive, balanced t(3;14) translocation in a patient with primary pulmonary lymphangiectasia. *D. N. Finegold[1,2], A. Smith[2], M. Sathanoori[1,2], M. C. Seleme[1,2], S. Li[2], U. Surti[1,2], M. Kimak[1], S. Bertera[1,2], R. E. Ferrell[1], R. D. Nicholls[1,2]* 1) University of Pittsburgh, Pittsburgh, PA; 2) University of Pittsburgh Medical Center, Pittsburgh, PA.

Primary pulmonary lymphangiectasia (PPL) is a rare disorder of infancy and childhood caused by an abnormal dilatation of the lymphatics of the lung. The cause of PPL is unknown. A child came to our attention that failed to initiate breathing at birth, was resuscitated, and was subsequently diagnosed with PPL without other anomalies. A karyotype revealed a balanced translocation 46,XY,t(3;14)(q27;q11.2), not present in either parent. We developed a novel methodology termed hybrid SNPing to resolve the breakpoints, by applying high density single nucleotide polymorphism (SNP) genotyping to human-mouse somatic cell hybrids retaining each translocation chromosome. Hybrid SNPing takes advantage of the differential hybridization and detection of human and mouse DNA to the SNP probes on the chip. The human SNPs on both recombinant chromosomes are identified within close proximity to the breakpoint while the rodent DNA typically results in no calls. This technique allowed us to rapidly resolve the breakpoints to within 4-5 kb and then easily fully identify the sequence across both breakpoints with PCR and DNA sequence analysis. Although the t(3;14) translocation did not directly interrupt any gene, the breakpoints localize to conserved non-coding sequences between *SALL2* (a putative oncogene) and *METTL3* in chromosome 14q11.2 and 5 of *BCL6* (a known oncogene in diffuse large B-cell lymphoma) in 3q27.3. Balanced translocations within intron 1 of *BCL6* and 5 of the gene are described as causal major and alternative breakpoints, respectively, in B-cell lymphoma. SNP genotype analysis, FISH, and quantitative dosage genomic PCR also identified genome-wide copy number variation (CNV) in this patient with duplication CNV in chromosome 9p24.1 and 16p11.2, and deletion CNV in 1q31.3. These CNV may be incidental or contribute to the PPL phenotype of this patient. Due to the *de novo* origin of the translocation, we suggest that genes flanking one or both breakpoints may be deregulated and potentially causal in PPL.

Integrated Analysis of Genetic and Proteomic Data Identifies Biomarkers Associated with Adverse Events Following Smallpox Vaccination. *D. Reif[1], A. Motsinger-Reif[2], B. McKinney[3], M. Rock[4], J. Crowe[4], J. Moore[5]* 1) Environmental Protection Agency, NC; 2) North Carolina State University, NC; 3) University of Alabama Birmingham, AL; 4) Vanderbilt University, Nashville, TN; 5) Dartmouth College, NH.

Complex clinical outcomes, such as adverse reaction to vaccination, arise from the concerted interactions among the myriad components of a biological system. Therefore, comprehensive etiological models can be developed only through the integrated study of multiple types of experimental data. In this study, we apply this paradigm to high-dimensional genetic and proteomic data collected to elucidate the mechanisms underlying development of adverse events (AEs) in patients following smallpox vaccination. Since vaccination was successful in all of the patients under study, the AE outcomes reported likely represent the result of interactions among immune system components that result in excessive or prolonged immune stimulation. In the current study, we examined 1442 genetic variables (SNPs) and 108 proteomic variables (serum cytokine concentrations) to model AE risk. To accomplish this daunting analytical task, we employed the Random Forests (RF) method to filter out the most important attributes, then we used the selected attributes to build a final decision tree model. Importantly, RF is a natural approach for studying the type of gene-gene, gene-protein, and protein-protein interactions we hypothesize to be involved in development of clinical AEs. Combining information from previous studies on AEs with the genetic and proteomic attributes identified by RF, we built a comprehensive model of AE development that includes the cytokines ICAM-1 (CD54), IL-10, and CSF-3 (G-CSF), and a genetic polymorphism in the cytokine gene IL4. The biological factors included in the model support our hypothesized mechanism for the development of AEs involving prolonged stimulation of inflammatory pathways and an imbalance of normal tissue damage repair pathways. This study enhances and reinforces our working model of AE development following smallpox vaccination.

Synapsis and recombination in a 69,XXX fetus: implications for normal female meiosis. *A. Kashevarova[1], T. Hansen[1], T. Hassold[1], T. Naluai-Cecchini[2], E. Cheng[2]* 1) School of Molecular Biosciences, Washington State Univ, Pullman, WA; 2) Dept of OB/GYN, Univ of Washington, Seattle, WA.

Triploidy is a common chromosome abnormality, accounting for nearly 10% of miscarriages. The extra haploid set makes it useful for studying the consequences of dosage imbalance on synapsis and recombination (events occurring in utero in females) but surprisingly, nothing is known of this process in human triploids. We recently examined meiosis in a 20 week 69,XXX fetus, using antibodies against synaptonemal complex (SC) proteins SYCP1 and SYCP3 and the cross-over associated protein MLH1, CREST antiserum and chromosome 8 and 21 FISH probes, to investigate genome wide and chromosome-specific patterns of synapsis and crossing-over. In analyses of over 200 cells, we identified oocytes at each sub-stage of meiotic prophase. Those that reached pachytene were clearly abnormal, as no cells exhibited 23 fully synapsed trivalents. Nevertheless, some cells were able to proceed through the pachytene checkpoint controls, as evidenced by the presence of diplotene images. Thus, unlike mammalian males, where synaptic errors trigger meiotic arrest, human females appear less responsive to checkpoints monitoring meiotic synapsis. In analyses of genome-wide recombination levels,we found a surprising increase in the triploid fetus: the mean number of MLH1 foci/cell was 109, 1.5 fold that of the normal female value of 75. This suggests that recombination levels respond in a dosage dependent manner to the availability of other meiotic reagents; e.g. to the level of SC proteins binding the three homologs. This was reinforced by analyses of individual chromosomes; e.g., chromosome 21. We identified cells with one of three chromosome 21 synaptic configurations - a bivalent and a univalent, a partially synapsed trivalent, or a completely synapsed trivalent. The first of these (bivalent/univalent) was associated with a normal level of MLH1 foci, while the latter two had significantly increased (1.5 fold) levels. Thus, the presence of even partial synapsis of all three chromosomes was associated with an increase in crossing-over, demonstrating a direct relationship between synapsis and recombination.

Electronic Medical Records Linked to DNA: A Valuable Resource for Large-Scale Genetic Association Studies.

D. Crawford[1,5], M. D. Ritchie[1,5], J. C. Denny[2], A. Havens[3], J. Weiner[3], J. M. Pulley[6], M. A. Basford[6], D. R. Masys[2], D. M. Roden[3,4,6], J. L. Haines[1,5] 1) Ctr Human Genetics Res, Vanderbilt Univ, Nashville, TN; 2) Department of Biomedical Informatics, Vanderbilt University, Nashville, TN; 3) Department of Medicine, Vanderbilt University, Nashville, TN; 4) Department of Pharmacology, Vanderbilt University, Nashville, TN; 5) Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN; 6) Office of Personalized Medicine, Vanderbilt University, Nashville, TN.

Large scale DNA databanks linked to electronic medical records (EMR) have been proposed as an approach to generating large, diverse cohorts, given their potential for rapid sample collection and availability of richly detailed longitudinal data. However, it is unknown whether the phenotypes can be reliably extracted with adequate precision, and whether the associated DNA can be analyzed to establish associations. The Vanderbilt DNA Databank approaches these challenges by (1) using DNA collected from discarded blood in an opt-out model, (2) linking to a de-identified derivative of the EMR. The DNA Databank accrues ~700 new samples/week (38,103 as of 6/12/08). As an initial test of the methodology, we used natural language processing techniques and billing code queries on the first 10,000 records to extract European-American cases and controls for rheumatoid arthritis (RA; n=170 cases /701 controls), multiple sclerosis (MS; n=66/1857), Crohns disease (n=116/2643), and atrial fibrillation (AF; n=168/1695). 13 disease-associated, replicated SNPs were extracted from genome-wide association studies (GWAS) and tested for association in these samples. At least one associated SNP for each disease was replicated in our population [RA (rs6457617, $p<0.0001$, OR=1.95); MS (rs3135388, $p<0.0001$, OR=3.3); Crohns (rs17234657, $p<0.001$, OR=1.78; rs1000113, $p=0.028$, OR=1.63; rs17221417, $p=0.02$, OR=1.4); atrial fibrillation (rs2200733, $p=0.04$, OR=1.4)]. These data suggest that the DNA and the data extracted from an EMR system are of sufficient quality and validity for genomic research, providing support for such resources as invaluable for genotype-phenotype hypothesis generation and confirmation.

Association Studies of Positional Candidate Genes to Total Cholesterol Levels on Chromosome 5q31. *B. S. Sutton[1], S. G. Gregory[1], C. Haynes[1], D. R. Crosslin[1], S. C. Nelson[1], J. J. Connelly[1], K. Dehghanpisheh[1], S. G. Watson[1], D. Seo[3], J. M. Vance[3], C. J. H. Jones[4], D. C. Crossman[5], V. Mooser[6], C. B. Granger[2], P. J. Goldschmidt-Clermont[3], W. E. Kraus[2], E. R. Hauser[1], S. H. Shah[1,2]* 1) Center for Human Genomics, Duke University, Durham, NC; 2) Department of Medicine, Duke University, Durham, NC; 3) Miller School of Medicine, University of Miami, Miami, FL; 4) University of Wales, Cardiff, UK; 5) University of Sheffield, Sheffield, UK; 6) GlaxoSmithKline, Philadelphia, PA.

Coronary artery disease (CAD) and its associated phenotypes are widely accepted to be driven by environmental and genetic influences. We have previously shown linkage to total and low density lipoprotein (LDL)-cholesterol to chromosome 5q31 in early-onset CAD families (GENECARD). Fine-mapping was performed using 1409 SNPs covering a 50 Mb region (117 Mb to 167 Mb). Genotyping was performed on two CAD datasets including 272 GENECARD families (N=741) and 1975 case/control samples from an independent CAD cohort (CATHGEN). Lipoprotein measurement was performed on all individuals using NMR spectroscopy and used for quantitative trait analysis. Our trait of interest was very small LDL particle size (VSP) given its high correlation with LDL cholesterol. Human aortas (N=164) were used for association with atherosclerosis as determined by Sudan IV staining and raised lesion assessment. Logistic regression was used for case/control association and APL (Association in the Presence of Linkage) was used for family based association. Sixteen genes were significantly associated ($P<0.05$) with VSP in both GENECARD families and CATHGEN case/control datasets. Of these, 10 genes were also significantly associated ($P<0.05$) with CAD in GENECARD, CATHGEN, and with atherosclerosis in the aortas. Furthermore, four of these genes (TRPC7, CSS3, SPOCK and FSTL4) contained SNPs with evidence of linkage (highest LOD=2.0, 1.8, 1.9, and 1.7 respectively). Thus, based on the convergence of multiple datasets and independent statistical analyses, we have maximized the identification of true associations and are currently evaluating the genetic contributions of the identified genes.

The Duke Personal Variome Project: Analysis of Psychosocial Implications Associated with Whole-Genome Scans. *S. B. Haga, J. M. O'Daniel* Inst Genome Sci & Policy, Duke Univ, Durham, NC.

Whole-genome scans have moved from a solely research technology to a commercial laboratory service offered directly to consumers, revealing information about disease risks and genetic ancestry. However, the related psychosocial implications have not yet been studied. We conducted a pilot study, the Duke Personal Variome Project (PVP), to begin to explore the psychosocial and health behavioral impact associated with testing and disclosure of individual variomes (an assembled catalogue of genetic variations). One million SNPs were tested utilizing the Illumina Infinium whole-genome BeadChip array. SNPs highly associated with a disease were assessed for independence on the basis of linkage disequilibrium. This resulted in a list of variants significantly associated with 31 unique conditions for which individual reports were compiled for each participant. Fourteen subjects with advanced training in genetics were recruited; the median age was 41y and 71% have biological children. A stratified consent was used which required an initial consent to study participation prior to testing and a second consent prior to results disclosure. Participants could indicate their preference for non-disclosure of data associated with particular conditions (e.g., breast cancer) as well as indicate the level with which their data could be further analyzed and reported for study publications. To explore the expected and actual impact of testing, participants underwent semi-structured interviews at pre- and post-test and 3-months follow-up. In addition, they completed a brief survey at 1-week and 3-months post-disclosure. Personal curiosity was a primary motivation (93%) and expectations were guarded. Predictions for testing to reveal a personal disease risk ranged from 10 to 100% (median = 85%). Only 14% believed they would most likely change current health behaviors based on the data; 50% reported they may change behaviors and 36% reported they would not based on several factors. Participants will be followed up to one year post-disclosure. The PVP is one of the first studies to explore psychosocial and behavioral implications that may arise from whole-genome scans.

Characterization of BBS3 (ARL6) isoforms and identification of a distinct role in vision for BBS3 long in mouse and zebrafish. *P. R. Pretorius[1,7], L. M. Baye[2,7], R. F. Mullins[3,7], C. C. Searby[4,7], D. Y. Nishimura[4,7], K. Bugge[4,7], B. Yang[5,7], E. M. Stone[3,4,7], D. C. Slusarski[2,7], V. C. Sheffield[4,6,7]* 1) Genetics Program; 2) Dept Biology; 3) Dept Ophthalmology; 4) HHMI; 5) Dept Obstetrics and Gynecology; 6) Dept Pediatrics; 7) Univ Iowa, Iowa City, IA.

Hundreds of individually rare, but collectively common Mendelian disorders cause blindness. One of these disorders is a heterogeneous syndromic form of retinal degeneration, Bardet-Biedl Syndrome (BBS). Typically, individuals with BBS experience central vision loss during childhood or early adolescence and are blind by the third decade of life. Our lab utilizes both the mouse and zebrafish to unravel the mechanism underlying retinal degeneration seen in BBS. We identified two transcripts of BBS3, a member of the Ras family of small GTP-binding proteins. *Bbs3S*, the common Bbs3 transcript is expressed in all mouse tissues examined, while *Bbs3L* was predominantly expressed in the eye. In the zebrafish, *bbs3S* is ubiquitously expressed at all stages of development, while *bbs3L* expression coincides with photoreceptor development. Moreover, immunohistochemical analysis of BBS3 reveals protein localization to both ganglion cells and photoreceptor cells of human, mouse and zebrafish retinas. The eye-specific expression of the BBS3L isoform will facilitate the dissection of BBS function in the retina, independent of alterations to other tissues. To this end, a Bbs3L knockout mouse has been generated and histological analysis is underway to evaluate retina structure. Interestingly, using an antisense morpholino to transiently knockdown bbs3 function in the zebrafish, different morphological phenotypes arise when *bbs3S* is targeted compared to *bbs3L*. A morpholino that targets both isoforms results in defects to the ciliated Kupffers Vesicle, melanosome transport delays and cone vision impairment. Defects to the Kupffers Vesicle, as well as a delay in retrograde transport are both cardinal features of BBS phenotypes (*bbs1-12*) in zebrafish. Consistent with an eye specific function *bbs3L* morphant embryos have normal Kupffers Vesicle formation and melanosome transport, but show cone vision defects.

Early Siberian Maternal Lineages in the Tubalar of Northeastern Altai Inferred from High-Resolution Mitochondrial DNA Analysis. *R. Sukernik, I. Mazunin, E. Starikovskaya, N. Volodko, N. Eltsov* Laboratory of Human Molecular Genetics, Institute of Cytology and Genetics SB RAS, Novosibirsk, Russian Federation.

At the hight of the last glaciation (~18 kya) Siberians were confined to the southern strongholds, which were areas of continuous occupation, and where immediate ancestors of the Uralic, Kettic and Altaian language groups differentiated. To better understand the evolutionary relationships between the earlier and contemporary Siberians, we focused on the northern Altaic prehistory preserved in the mtDNA diversity of the Tubalar, until recently representing a typical hunting-gathering population. The present study includes 139 Tubalar. All mtDNAs were subjected to high-resolution SNP analysis, followed by complete sequencing of selected mtDNA samples. We showed that the core of the Tubalar genetic makeup proved to be a mixture of west (H8, U4b, U5a1, and X2e) and east Eurasian (A and B1) haplogroups derived from macrohaplogroup N, and Siberian derivatives of the macrohaplogroup M identifiable by subhaplogroup-specific mutations. For example, among the 36 Tubalar mtDNA samples that belong to haplogroup D, 10 (28%) harbored diagnostic markers of the subhaplogroup D3a2a shared with the Chukchi and Eskimos. This finding verified at the complete sequence level we attributed to ancient link between early Siberians, who underwent pronounced differentiation in the Altai-Sayan region, and some of the Eskimo tribes. A comparison of the mtDNA data generated through the course of this study with published complete sequences has contributed essentially to parsimonious phylogenetic structure of mtDNA evolution in west Siberia. Specifically, northeastern Altai appears to be a good candidate for the ancestral homeland of the haplogroup U4b, which is apparently ancient European. For some haplogroups, such as X2e, the relatively recent arrival to the Altai region is more likely.

Analysis of fat mass and obesity associated (FTO) gene variants with measures of obesity and glucose homeostasis in the IRAS Family Study. *M. R. Wing[1], J. Ziegler[1], C. D. Langefeld[1], S. M. Haffner[2], J. M. Norris[3], M. O. Goodarzi[4], D. W. Bowden[1]* 1) Wake Forest University School of Medicine, Winston Salem, NC; 2) University of Texas Health Sciences Center at San Antonio, San Antonio, TX; 3) University of Colorado Health Sciences Center, Denver, CO; 4) Cedars-Sinai Medical Center, Los Angeles, CA.

Studies focusing on European-derived populations have provided evidence that FTO variants are associated with adiposity measures such as body mass index (BMI). Replication studies in other ethnicities have had mixed success. The goal of this study was to evaluate whether FTO SNPs were associated with adiposity measures, including computed-tomography derived measures of visceral and subcutaneous adipose tissue (VAT; SAT), and glucose homeostasis (frequently sampled glucose tolerance test derived measures of insulin sensitivity and acute insulin response, and fasting glucose and insulin) in the Insulin Resistance Atherosclerosis Family Study cohorts. SNPs covering FTO intron 1, including those prominent in the literature (rs9939609, rs8050136, rs1121980, rs17817449, rs1421085, rs3751812), were genotyped in 1,424 Hispanics and 604 African Americans and analyzed using the variance component method implemented in SOLAR. In Hispanics, multiple SNPs were associated with BMI and SAT (p-values ranging from 0.001 to 0.033), and trending or associated with waist circumference (p-values ranging from 0.008 to 0.099). For glucose homeostasis measures, SNPs were associated with fasting insulin but consistent with other studies, after BMI adjustment no association was observed. In the African Americans, only rs8050136 and rs9939609 were trending or associated with BMI, waist, and SAT (p-values ranging from 0.011 to 0.058). This genetic analysis adds to the growing evidence that FTO SNPs predispose individuals to obesity by increasing overall fat mass. In this study FTO does not contribute to differential fat deposition in a specific site and does not influence glucose homeostasis when adjusted for the contribution of BMI. Evidence of association in Hispanic Americans with measures of adiposity is fairly consistent, but more marginal in African Americans.

Fertility and Fitness Among the Deaf. *S. Blanton[1], A. Burt[1], W. Nance[2], A. Pandya[2], K. Welch[3], V. Norris[3], K. Arnos[3]*
1) Univ Miami, FL; 2) VA Commonwealth Univ, Richmond; 3) Gallaudet Univ, Washington DC.

The acquisition of sign language is perhaps the most important factor that can improve the genetic fitness of the deaf. Although their fitness was generally quite low in Europe prior to the introduction of sign language and the establishment of schools for the deaf nearly 400 years ago, there are several recent examples of population isolates in which indigenous sign languages are used within extended families to allow deaf and hearing family members to communicate with one another. This integration into the community diminishes the effect of deafness on fitness. In order to obtain contemporary estimates of the genetic fitness of the deaf in the US, we collected pedigree data as part of a larger epidemiologic study of deaf probands who are alumni of Gallaudet University. Information on the mating type and number and hearing status of children of both probands and their hearing and deaf siblings was collected. Data was obtained on a total of 686 deaf individuals and 602 hearing siblings; all individuals were at least 35 years old. Data was further subdivided into four groups based on marital status and gender. The average age of the four groups of adults ranged from a low of 50 (single deaf males) to 58 (married hearing males). The proportion of deaf adults who marry is similar to hearing adults (.83 vs .85). Among married individuals, the fertility of deaf individuals is less than their hearing siblings (p=.005), with a greater difference between females (p=.006). Genetic fitness has classically been measured as the ratio of the overall fertility of affected subjects to their unaffected siblings. While the fitness of deaf individuals in our study is significantly reduced (p=.002), it is not substantial. Our fitness estimates are substantially higher than contemporary values obtained from Sichuan (0.6) and Shanghai (0.78) China. We have also stratified fertility rates among deaf individuals and their hearing siblings by mating type; while HxH matings have the highest average number of children (2.24), DxD matings produced more children (2.11) than DxH matings (1.85), suggesting that fertility among the deaf is influenced by multiple factors.

Genetic variation in tribes of Eastern and North-Eastern India: inference from distribution of Y-chromosomal polymorphisms. *M. Borkar, F. Ahmed, F. Khan, S. Agrawal* Medical Genetics, SGPGIMS, Lucknow, India.

Background: The Eastern and North-Eastern region of Indian subcontinent is mainly inhabited by tribes belonging to Austro-Asiatic and Sino-Tibetan linguistic family. The different migratory episodes influenced their genetic structure. **Objectives**: To investigate the paternal population history of total 607 individuals from nine populations of Eastern and North-Eastern tribes from India. **Methods**: 34 binary markers and 17 short-tandem-repeat loci from the non-recombining part of the human Y chromosome were analyzed by RFLP, Sequencing and Genescanning. **Results**: The tribal populations were characterized by a diverse set of 15 haplogroups. A single haplogroup (O-M175) accounts for ~70% of North-East Indian Y chromosomes. The Neighbour joining tree drawn to analyze the phylogenetic relationship of tribal populations with different world populations revealed a strong genetic affinity of Austro-Asiatic and Sino-Tibetan populations with Orientals inhabiting in East-Asia. The results were compared with different castes and tribal populations of India to draw the complete phylogeny picture of studied tribes. The results support the hypothesis that the upper castes from North India are descendant of European populations. On the basis of Y-chromosomal STR haplotypes the Tibeto-Burman tribals can be differentiated from Austro-Asiatic tribals but both group showed considerable genetic similarity suggesting that they may have shared a common habitat in East-Asia. AMOVA analysis suggests that the Indian lower castes are genetically more similar to the tribal populations, than to the higher castes. Our results support that there was mostly male-mediated migration of the population to India, where they admixed with local females. The overall haplotype diversity for the 17 Y-STR loci was 0.9998, and the discrimination capacity was 0.9695. These results are compared with those observed in worldwide populations at both the locus and the haplotype level. **Conclusion**: The phylogeographic profile demonstrated different clinal patterns that may indicate source, direction, and relative timing of different waves of dispersals and expansions involving these nine populations.

Linear clinical progression independent of age of onset in Niemann-Pick Disease, type C. *N. M. Yanjanin[1], J. I. Vélez[1], A. Gropman[2], K. King[1], C. C. Brewer[1], B. Solomon[1], W. Pavan[1], M. Arcos-Burgos[1], M. C. Patterson[3], F. D. Porter[1]* 1) NICHD, NHGRI, NIDCD or CC of NIH, DHHS, Bethesda, MD; 2) CNMC, Washington, DC; 3) Mayo Clinic, Rochester, MN.

Niemann-Pick Disease, type C (NPC) is a neurodegenerative storage disorder characterized by ataxia, dystonia, dementia, and vertical ophthalmoplegia. The heterogeneous clinical nature and variable age of onset confounds the characterization of potential biomarkers, and the absence of an accepted biomarker is an impediment to the development of therapeutic trials. Thus, we developed a clinical severity scale to correlate with potential biomarkers, and monitor disease progression. Clinical data were collected from 18 current patients and retrospective data were extracted from the records of 19 patients. Symptoms in 9 major (scored 0-5: ambulation, cognition, eye movement, fine motor, hearing, memory, speech, seizures, swallowing) and 8 minor domains (scored 0-3: ABR, behavior, gelastic cataplexy, hyperreflexia, incontinence, narcolepsy, psychiatric, respiratory) typically noted in a medical history were scored. Interestingly, both cohorts showed a linear increase in severity. Cross-sectional analysis of 18 current patients showed a linear increase (r^2=0.64, p<0.0001) with a mean progression of 1.40.3 points per year. Longitudinal chart review of 19 patients showed that although age of onset varied significantly, the rate of progression (mean=1.90.2 points per year) was independent of age of onset and similar in 18/19 patients. Combining the data from both cohorts, progression could be mathematically modeled: $Åœt_0+x=Åœt_0+1.87x$; where $Åœt_0$ is the initial score and $Åœt_0+x$ is the predicted score after x years. The standard error and the 95% CI for the slope were 0.18 and 1.6-2.3 respectively, allowing for power estimates to be obtained. Our observation that disease progression is similar in patients and independent of age of onset is consistent with a biphasic pathological model for NPC and does not support a model of slower progression in later onset cases. In addition, this scale may prove useful in the characterization of potential biomarkers, and as an outcome measure to monitor disease progression.

Long-term Rescue of a Lethal Murine Model of Methylmalonic Acidemia using AAV 8 Mediated Gene Therapy.
R. J. Chandler, C. P. Venditti Genetic Disease Research Branch, NHGRI/NIH, Bethesda, MD.

Methymalonic acidemia (MMA), a severe organic acidemia, is caused by deficient activity of the ubiquitous mitochondrial enzyme methylmalonyl-CoA mutase (MUT). MMA patients exhibit increased methylmalonic acid levels in the plasma, urine and CSF and display a clinical phenotype of lethal metabolic decompensation, growth retardation, renal failure and metabolic strokes. To assess the potential of genetic therapy for MUT MMA, we employed a mouse model of MMA that produces no detectable Mut transcript or protein. AAV 8 CBA-Mut was injected directly into the liver of newborn Mut-/- pups. Currently, 28 out of the 29 Mut-/- mice injected with 1 or 2x1011GC of AAV 8 CBA-Mut are alive beyond DOL 90 with some treated Mut-/- mice older than 200 days. All the untreated mutants (n=21) perished before DOL 72. The treated Mut-/- mice are thriving and indistinguishable from their wild-type (WT) littermates. AAV 8 CBA-Mut treated Mut-/- mice achieved body weights comparable to controls while untreated mutants experienced post-natal growth retardation and reached only 40% of the weight of the WT. Plasma methylmalonic acid levels in the treated mutant mice on an unrestricted diet were significantly reduced compared to uncorrected animals, indicating that substantial Mut enzymatic activity was restored after AAV therapy. At DOL 90 the liver from a treated Mut-/- mouse had WT levels of Mut protein by Western blot analysis. These experiments provide the first evidence that gene therapy has clinical utility in treatment of MMA and support the development of gene therapy for other organic acidemias.

Isolated autosomal recessive nail dysplasia with pachonycia and onycholysis in a consanguineous Pakistani family. A novel form of nail dysplasia that maps to chromosome 8. *A. Fröjmark[1], M. Entesarian[1], S. Nawaz[2], J. Schuster[1], J. Klar[1], M. Rasool[2], M. Tariq[2], I. Ahmad[2], S. Baig[2], N. Dahl[1]* 1) Genetics and Pathology, Uppsala University, Uppsala, Sweden; 2) Health Biotechnology Division, National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad, Pakistan.

Developmental nail abnormalities are clinically and genetically heterogeneous, being small and rare subgroups. We have investigated a consanguineous Pakistani family with four members affected by autosomal recessive nail dysplasia. Affected individuals present with claw-like pachonychia, nail clubbing and onycholysis of both finger- and toenails but no other ectodermal symptoms. Hair, skin and teeth were found normal and affected individuals reported normal sweating. We performed a genetic analysis on the family using a 250K SNP array (Affymetrix) as well as microsatellite markers. One genomic region spanning more than 90 homozygous SNPs on chromosome 8 was found to be shared by affected individuals. Linkage analysis with chromosome 8 microsatellite markers revealed a maximum LOD score of 2.96 (= 0). Recombination events restricted the candidate region to 18 Mb which spans approximately 100 genes. We are now in the process of sequencing candidate genes in search for mutations.

A homozygous deletion of 8q24.3 including the NIBP gene associated with severe delay, agenesis of corpus callosum and facial dysmorphism. *A. Koifman[1,4], A. Feigenbaum[1], B. Weimin[2], LG. Shaffer[3], D. Chitayat[1,4]* 1) The Hospital for Sick Children, Division of Clinical and Metabolic Genetics, University of Toronto, Toronto, Ontario, Canada; 2) Departments of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas, USA; 3) Signature Genomic Laboratories, Spokane, Washington, USA; 4) Mount Sinai Hospital, The Prenatal Diagnosis and Medical Genetics Program, Department of Obstetrics and Gynecology, University of Toronto, Toronto, Ontario, Canada.

We have identified by microarray-based comparative genomic hybridization analysis (aCGH), a homozygous deletion of 8q24.3 in a patient with clinical manifestations of severe global developmental delay, agenesis of corpus callosum and facial dysmorphism. The deletion was inherited from asymptomatic, consanguineous parents, each of them being heterozygous for the same deletion. The only gene known to map to this segment is the NIBP gene and so far no clinical manifestation have been found in association with this gene mutation in homozygous or heterozygous state in humans. Our findings suggest that a mutation in the NIBP gene results in an autosomal recessive condition with multiple abnormalities including agenesis of the corpus callosum, severe developmental delay and specific facial dysmorphism . In addition, the child inherited a 781 Kb deletion on 4q32.2 from the mother that contains the SPOCK3 gene. We present evidence that this heterozygous deletion is likely to be noncontributory to the phenotype.

11q Terminal Deletion in a Patient with Jacobsen Syndrome: Molecular Characterization. *L. D. Kulikowski, T. B. De Maio, J. F. S. Franco, F. T. S. Bellucco, S. I. Nogueira, D. M. Christofolini, A. N. X. Pacanaro, R. S. Guilherme, A. B. A. Perez, M. I. Melaragno* Universidade Federal de Sao Paulo, Sao Paulo Brazil, Sao Paulo, Brazil.

Jacobsen syndrome is a contiguous gene disorder caused by a deletion in the long arm of chromosome 11. Common clinical findings include developmental delay, short stature, congenital heart defects, thrombocytopenia, genitourinary anomalies, pyloric stenosis and facial dysmorphology. We report on a male patient with the 11q terminal deletion with many of the features of Jacobsen syndrome. The karyotype of the patient is 46,XY,der(11)t(8;11)(q24.3;q23)pat. The deletion terminal was confirmed using a set of BAC-FISH probes for distal 11q including the subtelomeric RP11-345O3 probe, corresponding to position 134.2 Mb from 11p, that was absent in the der(11). Previous molecular analyses of 11q deletions have been performed on a small number of cases and have demonstrated that the deletions range in size from 7 Mb to greater than 20 Mb. All cases analyzed using molecular techniques demonstrated the presence of a terminal deletion with retention only of telomeric sequences at the end of 11q. The present case differs from the reported cases since there was no FISH signal for the specific 11q subtelomeric probe. The molecular characterization of the segment deleted in patients with terminal 11q deletion is important to localize disease-causing genes in this region. The deletion present in our patient includes JAM-3 gene, a candidate gene for causing several heart defects in Jacobsen syndrome, although he has only a heart murmur. Most of patients with del(11q) had a history of thrombocytopenia and/or abnormal platelet morphology indicative of Paris-Trousseau syndrome. There is strong evidence that the FLI-1 gene, a transcription factor that plays a role in megakaryopoiesis, is implicated in this syndrome. Interestingly our patient did not have giant platelets, suggesting that a second gene may contribute to this aspect of Paris-Trousseau phenotype. Apparently, the clinical picture of the Jacobsen syndrome is related to the molecular architecture, size and location of the deleted chromosome segments.

Linkage analysis of nonsyndromic cleft lip with or without cleft palate in a consanguineous Pakistani family. *E. Richards[1], S. Malik[2,3], B. Egbert[1], A. Ferguson[1], D. Wilcox[1], A. Lalwani[4], S. Naz[2], E. Wilcox[1]* 1) Dept Biol, Brigham Young Univ, Provo, UT; 2) School of Biological Sciences, Univ of the Punjab, Quaid-e-Azam Campus, Lahore, Pakistan; 3) Dept of Microbiology and Molecular Genetics, Univ of the Punjab, Lahore, Pakistan; 4) Dept of Otolaryngology, NYU School of Medicine, New York, NY.

Nonsyndromic cleft lip with or without cleft palate (NSCLP) is a heritable disease with a complex etiology. NSCLP is highly heterogeneous and loci identified so far only account for a small percentage of cases. To identify additional loci for NSCLP, we are studying large consanguineous families segregating NSCLP, an approach that has been shown to be effective in other gene-mapping studies. So far, we have performed linkage analysis on one Pakistani family segregating unilateral cleft lip (25 total samples with 7 affected individuals and 7 instances of consanguinity [4 documented and 3 undocumented]).

To find alleles segregating among the affected members of this family, SNP alleles for a portion of the samples were determined using Affymetrix GeneChip Human mapping 250K Nsp arrays. The resulting SNP data was screened in Alohomora [Bioinformatics 21(9): 2123] to remove problematic or uninformative genotypes. Data was formatted further by Mega2 [Bioinformatics 17(12): 1244-5] and parametric and nonparametric linkage (NPL) analyses were performed by Simwalk2 [Hum Hered 52:121-131]. These analyses yielded 17 regions with LOD and NPL scores >3.0. We then typed two additional highly heterogeneous STRs in each of these regions using fragment analysis. The STR alleles were added to the SNP data, and the 17 regions were reanalyzed. Linkage analysis with the additional markers eliminated 11 regions (reducing both LOD and NPL scores to <3.0) and further enhanced 4 regions (increasing LOD and NPL scores to as high as 3.9 and 3.4 respectively). No single allele exhibits simple dominant or recessive inheritance across all affected and unaffected individuals, suggesting that several loci work in concert to express the phenotype.

Potential Role of BRD2 Splicing in Juvenile Myoclonic Epilepsy. *H. M. Sanders[1,2], E. Shang[2,3], D. J. Wolgemuth[3,4,5,6], D. A. Greenberg[1,2,7]* 1) Psychiatry Department, Columbia University, New York, NY; 2) Division of Statistical Genetics, Columbia University, New York, NY; 3) Institute of Human Nutrition, Columbia University, New York, NY; 4) Department of Genetics and Development, Columbia University, New York, NY; 5) Department of Obstetrics and Gynecology, Columbia University, New York, NY; 6) Herbert Irving Comprehensive Cancer Center, Columbia University, New York, NY; 7) New York Psychiatric Institute, Columbia University, New York, NY.

Juvenile Myoclonic Epilepsy (JME) is associated with SNPs and a microsatellite marker (GT-repeats) in the gene BRD2. An evolutionarily conserved, alternatively spliced exon (2a) is located in the same intron (Intron 2) as the associated microsatellite. We have proposed that the sequences of the intronic region between exons 2 and 3 may influence the splicing of the alternative exon 2a and hence, JME susceptibility. A four-basepair polymorphism, GTAA, allowed us to test if the intronic region sequence influences splicing. The GTAA polymorphism is found upstream from the GT-repeat region. We created two constructs of Exon2-Intron2-Exon3 of BRD2 from a human DNA sample, one with the GTAA-containing allele and one without. The microsatellite allele in both constructs was identical. We transfected mammalian expression constructs into 293T cells and examined splice products. Northern blot analysis of total RNA demonstrated differences in splicing of the two alleles. While products included both normally spliced (Exon2-Exon3) and alternatively spliced products (Exon2-Exon2A-Exon3), we also saw partially spliced products in greater proportions from the allele with the GTAA polymorphism than from the allele without. Complementary DNA sequencing showed that a portion of Intron2 was in the partially spliced product. This partially spliced product may reflect aberrant or inefficient splicing as a function of intronic length or structure. We propose that the presence or absence of this GTAA polymorphism and length of the microsatellite, may affect the function of BRD2 and play a role in the development of JME.

CCRaVAT - Case/Control Rare Variant Analysis Tool. *R. W. Lawrence, A. P. Morris, M. I. McCarthy, E. Zeggini, WTCCC* WTCHG, Oxford University, UK.

With the recent plethora of common variants found to be associated with complex diseases there is a realization that rare-variants may contribute a large part to their genetic content. This is true for type 2 diabetes (T2D) which has numerous associated genes but a relatively small cumulative lambda-s of 1.07. The available sample sizes, although sufficient for well-powered studies on common variants, do not provide sufficient power for single-point analysis of rare variants when effect sizes are small to moderate. We have developed rare variant analysis software which allows the analysis of low MAF SNPs by pooling rare variants within defined regions and treating them as a single super locus. Gene regions are defined as sequence 50kb either side of the transcriptional start/stop sites. For each region a 2x2 contingency table is constructed containing: (1) number of cases carrying at least one rare variant; (2) number of cases not carrying a rare variant; versus (3) number of controls carrying at least one rare variant; (4) number of controls not carrying a rare variant. Differences in frequency between rare variant carrying cases and controls are tested using Pearson's chi-squared test (or Fisher's exact test where required). Using this method we calculated P-values for all genes across the genome in the Wellcome Trust Case Control Consortium (WTCCC) type 2 diabetes (T2D) dataset. We found six gene regions in which rare variants of MAF0.01 were associated with T2D with $P10^{-4}$. The three strongest signals observed were: BMP2K (chr4, $p=2.9x10^{-6}$, two rare variants carried by 28/1821 cases and 9/2922 controls); LOC391845 (chr5, $p=3.7x10^{-6}$, six rare variants, carried by 26/1903 cases and 7/2913 controls) and CPN1 (chr10, $p=4.7x10^{-5}$, two rare variants, carried by 14/1921 cases and 1/2932 controls). Of the regions showing greatest significance there was a clear tendency for cases to have a higher proportion of rare variants compared to controls (5 of 6 regions with $P10^{-4}$ had higher proportions of rare variants in cases, binomial test p=0.11). We are following up regions that appear to be significantly different in rare variant content between T2D cases and controls.

Evaluation of gene expression patterns associated with genomic instability in colon cancer. *C. B. Wiese, B. Lomenick, T. A. Carver, M. J. Kovach* Biological & Environmental Sci, Univ. of Tennessee-Chattanooga, Chattanooga, TN.

Genomic instability is a molecular feature common to many cancers. Two types of genomic alterations associated with cancer are variability in microsatellites and global changes in DNA methylation status. The purpose of this study is to evaluate whether genes in the molecular pathways of cancer are subject to gene regulation by microsatellite repeat variability. We propose that microsatellite repeat elements represent a normal, but as yet uncharacterized mechanism of gene regulation in which polymorphisms in microsatellites function to control the local chromatin structure and mRNA secondary structure. We predict that microsatellite variants generate alternate secondary structures important in the recruitment of transcriptional proteins that directly influence the rate of transcription, as well as affect transcript stability. We also predict a relationship between the DNA structure induced by microsatellite variability and the methylation status of CpG islands, presenting an indirect mechanism by which genomic instability influence transcription. To examine the effect of microsatellite variability on gene expression, DNA from 14 colon cancer cell lines was characterized for 22 microsatellite elements in 7 genes associated with colorectal cancer. Preliminary evaluation indicates that microsatellites near the 5 and 3 ends of the transcripts exhibit a higher degree of variability; regions known to influence mRNA abundance. Secondly, the methylation status of CpG islands within these genes was analyzed by combined bisulfite restriction analysis (COBRA). Methylation varied depending on the functional nature of the gene. For example, the angiogenesis inhibitor *THBS1* is primarily unmethylated (mean: 8.6% methylation) except for four RER+ cell lines. Cell line LS411N shows 98% methylation and the other three cell lines show 15% methylation. However, *STK11* shows partial methylation for all the cell lines with a mean of 91.5% methylation for the cancer cell lines and 81% methylation for the normal colon control. The increase in methylation suggests that transcription of this tumor suppressor gene is repressed in these cancer cell lines.

Analysis of genetic origins of canine MEN 2-like phenotypes. *A. A. Kovinich Smith[1], C. Gibson[2], J. Keating[2], J. Alroy[2], A. S. Tischler[3], L. M. Mulligan[1]* 1) Dept Pathology and Molecular Medicine, Queen's Univ, Kingston, ON, Canada; 2) Dept Biomedical Sciences, Tufts Cumming School of Vet Medicine, Grafton, MA, USA; 3) Dept Pathology, Tufts Univ, Boston, MA, USA.

The proto-oncogene *RET* encodes a receptor tyrosine kinase expressed in neural crest-derived cell types. Germline mutations in the human *RET* gene are responsible for multiple endocrine neoplasia type 2 (MEN 2). MEN 2 is an inherited cancer syndrome characterized by medullary thyroid carcinoma (MTC), and may also be associated with pheochromocytoma (PC) and other less common tumor and developmental phenotypes. Phenotypes similar to that of MEN 2 have been documented in species other than humans; however, corresponding similarity between genotypes has yet to be confirmed. We performed an *in silico* alignment of *RET* sequences from available species to identify areas of similarity in the kinase. Comparisons of *RET* sequences between human and animal species suggest strong evolutionary conservation and make these RET-orthologs likely candidates for involvement in the development of MEN 2-like phenotypes in other species. We identified a dog expressing MEN 2-like phenotypes including both MTC and PC upon post-mortem autopsy. To determine if *Ret* was involved in the development of these phenotypes, we analyzed the genomic DNA sequences corresponding to human *RET* exons 10-16 in this animal, since greater than 99% of all MEN2-*RET* mutations are found within these exons. Western blotting was used to confirm Ret expression in adrenal tumor tissue from this animal. No sequence variants were detected in tumor material from either MTC or PC, suggesting that the dog does not share the genetic etiology commonly causing human MEN 2 phenotypes. Our data suggest that, despite the evolutionary conservation and the involvement of RET in the development of MEN 2 in humans, the *Ret* proto-oncogene may not play a role in the development of similar phenotypes in other species.

Molecular analysis of translocation breakpoints in a novel t(1;9) in a patient with isolated cardiac defect. *S. Saitta, K. Pickering, A. Gotter, A. Hacker, M. Nimmakayalu, E. Goldmuntz, B. Emanuel* Childrens Hospital of Philadelphia, Philadelphia, PA.

Chromosomal rearrangements such as deletions, duplications, and translocations play a critical role in gene discovery. In particular, de novo balanced translocations that might interrupt a gene or its regulatory elements, have been especially helpful in understanding the etiology for many disorders. We studied a non-syndromic male child with isolated tetralogy of Fallot, a commonly encountered conotruncal cardiac defect. The patient was found to have an apparently balanced de novo translocation, t(1;9) (q32;q23.3). DNA from the patient was analyzed by high resolution SNP-based oligonucleotide array analysis (500K), and no evidence of pathologic copy number changes were detected either near the putative breakpoints, or in other regions of the genome. Following G-banding, the breakpoints were further mapped by FISH using BACs and fosmids from specific regions, and localized by breakpoint-spanning probes. To further narrow the regions, a cocktail of multiple PCR probes was also labeled and used for FISH. Finally, a series of primers were developed for long-range PCR to amplify across the putative junction. The amplified product, found only in the patient, shows end sequences that localize to chromosome 1, while the other end of the product shows sequence from chromosome 9 by BLAST analysis. While the breakpoint does not directly interrupt the coding sequence of a specific gene, its proximity to two developmental genes may result in their dysregulation. Ongoing sequence analysis of these genes, completed in 40 patients with isolated tetralogy of Fallot, has identified polymorphic variants, though potential pathologic variants have not yet been identified. Further molecular and structural analysis of the sequences involved in this novel translocation breakpoint and assessment of the genes are underway. Overall, the utilization of newer sequenced-based tools and techniques have helped to localize the breakpoints of this de novo balanced translocation and identify potential new regions of the genome involved in the etiology of cardiac defects.

Common polymorphisms in candidate genes influence breast cancer phenotype and prognosis. *D. Eccles[1,3], W. Tapper[2], V. Hammond[2,3], S. Gerty[3], P. Simmonds[3], A. Collins[2]* 1) AcUGeM, WCGS, Southampton Univ Hosp Trust, Southampton, United Kingdom SO16 5YA; 2) Bioinformatics GroupUniversity of Southampton, Duthie Building , SUHT SO16 6YD; 3) Cancer Sciences DIvision, Somers Cancer Research Building, Southampton University Hospitals, SO16 6YD.

Introduction: Genome wide association studies (GWAS) confirm the existence of common genetic variants that affect breast cancer risk(1,2) and there is evidence from these studies that common genetic variants may influence tumour phenotype. Breast cancer prognosis is linked to tumour characteristics and stage. In addition data from population registries suggest that prognosis may have a heritable component(3). We investigated SNPs in 30 candidate genes that might plausibly influence breast cancer phenotype and prognosis. Methods: We genotyped blood DNA from 1001 young onset (40 years at diagnosis) symptomatic breast cancer cases from the POSH study(4). 206 tagging SNPs were typed reporting on 30 candidate genes. We used the Cochrane Armitage Trend to test association of SNPs comparing phenotypic extremes and Kaplan Meier survival analysis and Cox proportional hazards to assess the effect of each genotype on survival. Results: SNPs in FGFR2 and TNRC9 were significantly more frequent in ER +ve compared with ER -ve breast cancer (ptrend = 0.000003) and (ptrend = 0.0014) whereas ATM SNPs were associated with ER -ve disease (ptrend = 0.0000985). The rs2228480 synonymous SNP in ESR1 was associated with significantly earlier distant relapse which appeared to be mainly due to an effect on tumour grade (p0.01), there was no association of ESR1 SNPs with ER status of breast tumours. SNPs in TNRC9 and MMP7 were associated with a poor outcome that was independent of all known clinical prognostic indices. Conclusions: These novel findings will need to be replicated in further studies but suggest inherited genetic variants may be either modulating the host response to malignant cell growth or predisposing to certain types of somatic mutation that drive malignant cell proliferation. 1. Nature 447,1087-93(2007); 2. Nat Genet 39, 870-874 (2007); 3.Breast Cancer Research 9(3)R39 (2007); 4. BMC. Cancer 7, 160 (2007).

Detailed behavior, language and neurological analysis of interstitial duplication 15q11.2-q12 autism patients. *L. T. Reiter[1], V. Brewer[2], J. T. Jabbour[3], J. Cleary[4]* 1) Dept Neurology, Univ Tenn HSC, Memphis, TN; 2) Dept Pediatrics, Univ Tenn HSC, Memphis, TN; 3) Memphis Neurology Practice, Memphis, TN; 4) Audiology & Speech-Lang Pathology, Univ Memphis, Memphis, TN.

Autism has proven a difficult disorder to evaluate both clinically and at the molecular level due to both a high degree of genetic heterogeneity and the influence of unknown environmental factors that may further alter phenotypes in these patients. This underlying genetic variation means that any given set of individuals with idiopathic autism analyzed clinically for detailed phenotypic variations in EEG, seizure status, MRI, serotonin levels or behavioral analysis may represent a combination of underlying molecular defects that may or may not be relevant to the next group of subjects examined. To better understand the minimal phenotypic elements of autism we chose to examine individuals with one of the simplest genotypes containing the fewest number of genes that consistently results in an autism phenotype. Individuals with interstitial duplications of chromosomal region 15q11.2-q12 present with autistic features and are one of the most common genomic lesion detected in autism. Using a battery of neuropsychological, language, neurological and genetic tests we examined 5 pediatric cases of 15q interstitial duplication. The most outstanding feature of these subjects is they appear to be mildly affected with one individual even scoring outside of the autism spectrum by ADOS and ADI evaluation. Although only one patient had a history of seizures, 3 out of 5 showed mildly abnormal brainwave activity by EEG. This activity appears to be sub-clinical and may dissipate with age. We also found a trend indicating that the verbal IQ may be equivalent or slightly higher than performance IQ, but the ability for these individuals to use language adaptively in daily life was limited. In general, these individuals also scored high for daily living skills on the Vineland test. In summary, this is the first step in defining features unique to duplication 15q autism patients which may illuminate genotype to phenotype correlations in autism.

The epigenetics of X-linked gene expression in monozygotic twins discordant for primary biliary cirrhosis. *M. Martin[1], A. Lleo[2,3], C. Selmi[2,3], JM. LaSalle[1]* 1) Medical Microbiology and Immunology, UC Davis School of Medicine, Davis, CA, USA; 2) Rheumatology, Allergy and Clinical Immunology, UC Davis School of Medicine, Davis, CA, USA; 3) Internal Medicine and Liver Unit, San Paolo School of Medicine, U of Milan, Milan, Italy.

Primary biliary cirrhosis (PBC) is a female dominant autoimmune chronic cholestatic liver disease with an expected genetic component to its etiology due to concordance in most monozygotic (MZ) twin sets. Previously we proposed an X-linked epigenetic component to explain the female prevalence of PBC. While most genes on the inactive X chromosome are silenced by promoter methylation, previous studies have shown that approximately 10% of X-linked genes exhibit variable patterns of inactivation in individual females. This study was designed to test the hypothesis that susceptibility to PBC arises from epigenetic modifications to an X-linked gene with variable silencing. Samples were from a unique cohort of MZ twin sets discordant and concordant (n=4 and n=1, respectively) for PBC. Expression levels of the 125 variably X inactivated genes was determined by RT-PCR analysis and two genes (*CLIC2* and *PIN4*) were identified and showed to be consistently down regulated in the affected twin of each pair. For promoter methylation analysis, DNA samples were bisulfite converted followed by PCR amplification, cloning and sequencing to determine methylation patterns and levels for each pair of discordant and concordant twins. Neither *CLIC2* or *PIN4* had promoter CpG islands and both showed partial methylation of CpG sties within 300 bp of the first exon. Promoter methylation of *CLIC2* showed no significant difference between samples, while *PIN4* methylation showed a positive correlation with expression in all samples. Interestingly, a discordant twin pair heterozygous for a genetic polymorphism that creates two additional CpG sites showed the best correlation between promoter methylation and expression of *PIN4*. These results suggest that expression of *PIN4* is down-regulated in affected versus unaffected PBC twins by a combination of genetic and epigenetic factors.

Admixture mapping identifies a putative chromosomal region for diabetic nephropathy (DN) in Mexican-Americans (MA). *M. F. Seldin[1], C. Tian[1], M. V. Pahl[2], K. Chen[1], H. Abboud[3], S. B. Nicholas[4], E. Ipp[5], N. Arar[3], F. Thameem[3], J. Tayek[5], S. Snyder[5], R. Kosoy[1], D. G. Ballinger[6], S. G. Adler[5], Family Investigation of Nephropathy and Diabetes (FIND)* 1) UC, Davis, Davis, CA; 2) UC Irvine, Irvine, CA; 3) UT San Antonio, San Antonio, TX; 4) UCLA, Los Angeles, CA; 5) Harbor-UCLA, Los Angeles CA; 6) Perlegen Sciences Inc., Mountain View, CA.

Epidemiological evidence suggests that there are differences in the prevalence of DN dependent on continental ancestry. MAs are disproportionately affected by DN, yet few genetic studies have been performed to identify the genetic contributions to DN in this population. Utilizing MA FIND participants with type 2 diabetes (T2DM) and DN as cases and geographically matched controls with T2DM without DN, a genome-wide admixture mapping scan was performed. All DN cases met criteria for nephropathy (0.3 mg albumin/24h, or random ACR 0.3 g/g) in subjects with T2DM. Non-DN controls were defined as subjects with DM duration >10 yrs, no first degree relative with known ESRD or CKD, and ACR <0.3 g/g. 664 cases and 490 controls also met quality control filters including >95% complete genotypes, <10% African admixture and database matching gender assignment. Admixture mapping was performed using the ADMIXMAP algorithms and applied a panel of 4300 European/Amerindian ancestry informative markers (AIMs) to estimate ancestry along each chromosome (parental population ancestry estimated using European and multiple Amerindian group genotypes). In an analysis including the gender, recruitment site, and diabetes duration as covariates, the maximum ancestry linkage peak was observed at rs721941 on chromosome 2 (2q37.1, 229.9 MB) and was linked to Amerindian ancestry (SCORE test = 4.3, nominal p value = 1.7e-5). Additional peaks, which also linked with Amerindian ancestry, were observed for regions of chromosome 1 (rs1564720, SCORE test = 3.13 and chromosome 14 (rs4267246, SCORE test 3.0). Only the chromosome 2 peak was significant by permutation testing (p <0.05 genome-wide). These findings have the potential to identify genes that predispose MAs to DN and to discern novel therapeutic targets.

Evidence for BMI mediated association of PREX1 SNPs with type 2 diabetes. *J. Lewis, N. Palmer, J. Ellington, J. Divers, C. Langefeld, B. Freedman, D. Bowden* Wake Forest University School of Medicine, Winston-Salem, NC.

In a dense SNP map analysis of the 20q12-13.13 type 2 diabetes mellitus (T2DM)-linked region on chromosome 20 we observed suggestive evidence of association with T2DM for SNPs near the phosphatidylinositol 3, 4, 5-triphosphate-dependent RAC exchanger 1 (*PREX1*) gene. To better understand this region (316 kb), we genotyped 31 additional SNPs in a cohort consisting of 300 diabetic patients enriched for end-stage renal disease (ESRD) and 310 healthy controls making a total of 59 SNPs genotyped across *PREX1*. Of these SNPs, 12 were significantly associated with T2DM with additive P-values ranging from 0.001 to 0.031. Six of these associations were replicated in a sample of 469 non ESRD diabetes cases and 442 controls (additive P-value range of 0.017-0.042). The combined analysis consisting of 769 cases and 752 controls resulted in the same 6 SNPs associated with T2DM in addition to 3 other SNPs leading to generally stronger evidence of association (additive P-value range of 0.001-0.041). To better understand the relationship between these SNPs, adiposity, and T2DM, we repeated the association test accounting for body mass index (BMI) resulting in only 2 SNPs remaining marginally associated with T2DM (additive P-value range of 0.030-0.044). Subsequently, we tested whether these SNPs were associated with BMI itself. In this analysis 10 SNPs were significantly associated with BMI with evidence of association comparable to that observed with T2DM (additive P=0.001-0.029). The test for association between BMI and these SNPs stratified by T2DM status identified 13 SNPs that were significantly associated with BMI solely in the case stratum with additive P-value ranging from 0.002 to 0.041 suggesting that BMI may mediate the effect of these SNPs on T2DM. A formal mediation analysis revealed that the effects of 6 SNPs are significantly mediated by BMI (30-40% mediated effect). These results suggest that SNPs near the *PREX1* gene may contribute to diabetes susceptibility, which is mediated through effects of adiposity in European Americans.

Methods and Discoveries Drawn from Twenty Genome-Wide Copy Number Variation Association Studies. *C. Lambert, J. Grover, I. Lake, G. Linse, G. Rudy* Golden Helix, Inc, Bozeman, MT.

With over a dozen ongoing collaborations with leading research organizations and access to a wealth of whole genome copy number variation (CNV) studies across multiple platforms, we have come across a number of challenges regarding data quality and analysis. To address these challenges we developed a number of methodologies in pre-processing data, including scaling quantile normalization and virtual array generation to thousands of samples for the calculation of log2 ratios against a reference population, detecting and correcting for batch effects and population stratification, optimal segmentation employing both single sample and multi-sample algorithms to detect CNVs, and whole genome CNV association analysis. We present methods and results using the various Wellcome Trust case/control studies (~2000 cases, ~1500 common controls) on Rheumatoid Arthritis, Bipolar Disorder, Coronary Artery Disease, Crohns Disease, Hypertension, Type I Diabetes, and Type II Diabetes.

One of the most persistent and challenging issues has been batch effect correction. With high genotyping call rates mostly unaffected by plate effects, the vast majority of research groups have either insufficiently randomized cases and controls on plates, or borrowed controls from other experiments. Unfortunately, CNV studies are very sensitive to batch effects. We have employed PCA-based approaches to mitigate the enormous confounding of CNV association studies by batch effects and population stratification. Further, in looking at over 20 different studies, we have seen particular regions of chromosome 7 and 14 persistently associated across a vast majority of diseases, regardless of genotyping platform. We have also found perhaps 30-40% of CNV associations are confirmed by past studies, whereas the remaining 60-70% represent novel findings. Corroborated CNV associations of note in the Wellcome Trust studies are regions in or near CHL1, PPP1R12B, SLC8A1, SMAD6 in Coronary Artery Disease, CHL1 in Bipolar Disorder, BTNL2 in Rheumatoid Arthritis, PTPRD in Hypertension, and GGTL4 in Type I Diabetes.

Evolution pathway of the oriental ALDH2 variant. *H. Li, J. R. Kidd, K. K. Kidd* Department of Genetics, School of Medicine, Yale University, New Haven, CT06520-8005, USA.

Mitochondrial aldehyde dehydrogenase gene (*ALDH2*) is one of the most studied human genes with thousands of relevant research reports. The East Asia specific allele *ALDH2*487Lys* produces an ALDH2 enzyme defective in oxidizing aldehyde into acetic acid; one effect is protection against alcoholism and other relevant disorders. Significant questions in human evolution studies are how and why this oriental ALDH2 variant arose and became frequent. We typed 41 SNPs and one STRP around the region of *ALDH2* in 45 world populations. Most of the polymorphisms showed high F_{st} values indicating high variation among the geographic regions. Some variants occur only in East Asia or the Americas, including the allele at rs671 defining *ALDH2*487Lys*. A long range haplotype network including all the typed polymorphisms drawn by the NETWORK program illustrates the diversification routes of *ALDH2*. The three-site haplotypes of rs10849970-rs671-rs10849971 best describe the evolutionary steps of the oriental *ALDH2* variant. The ancestral haplotype GGA occurs everywhere. The first mutational step to AGA appeared in sub-Saharan Africa. The second step to AGG started in Ethiopia and increased its frequency across Eurasia to East Asia. AGG subsequently evolved into two derived haplotypes, the oriental haplotype AAG and the American haplotype GGG. The long range haplotype test failed to detect any significant selection signals as high linkage disequilibrium was found in the region of *ALDH2*. Disease association studies of the *ALDH2*487His* allele reported protective effects for some diseases and increased susceptibility for others. Other variants in the region may be involved because of the very high LD. However, our analyses suggest that AGG without the derived allele of rs671 has been weakly selected for rather than AAG. The ALDH2 variants may have played very important roles in the demographic history of Asia.

A multifactor dimensionality reduction (MDR) approach for detecting epistasis in the presence of genetic heterogeneity. *R. Urbanowicz, P. Andrews, J. Moore* Computational Genetics Laboratory, Department of Genetics, Dartmouth College, Lebanon, NH.

Genetic epidemiology is confronted with a technology-induced explosion of information and the realization that the majority of common diseases are likely the result of multiple interacting factors. Genetic heterogeneity is an additional phenomenon that is expected to significantly increase the complexity of genetic architecture. Genetic heterogeneity refers to the existence of multiple genetic pathways that yield the same phenotypic outcome. The goal of the present study was to develop a computational method that is capable of detecting epistasis in the presence of genetic heterogeneity. To accomplish this goal we developed a modified version of the Multifactor Dimensionality Reduction method and software that was developed specifically to detect nonlinear gene-gene interactions in the absence of independent effects. The MDR approach uses a constructive induction algorithm to combine two or more polymorphisms in a way that captures interaction effects. Here, we introduce a data-driven constructive induction (DCI) MDR algorithm that systematically develops gene-gene interaction models in different partitions of the data. To evaluate this approach we designed a simulation study with cases and controls generated using a wide variety of different epistasis models with heritabilities ranging from 0.025 to 0.4 and sample sizes ranging from 400 to 3200. We combined data from different model in differing proportions (1:1, 1:2, 1:4) to simulate epistasis in the presence of genetic heterogeneity. While the power to detect both underlying epistatic models varies according to these dataset parameters, permutation testing indicates that DCI MDR can significantly improve power to accurately identify the two independent underlying epistasis models. This study represent a first step toward the development of computational algorithms that address multiple sources of complexity in the genetic architecture of human diseases.

Targeted exome sequencing of the NF1 (Neurofibromatosis Type 1) gene: sequence capture and enrichment (SCE) by a high-density oligonucleotide microarray followed by next generation sequencing (NGS). *L. Chou*[1], *R. Mao*[1,2] 1) ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT; 2) Department of Pathology, University of Utah Health Sciences Center, Salt Lake City, UT.

The recent introduction of instruments capable of generating millions of DNA sequence reads in a single run is rapidly changing the view of genetics. These instruments include: GS FLX (Roche 454), genome analyzer II (Illumina), SOLiD (ABI), and more. While implementation of these NGS instruments in a diagnostic laboratory remains in the future, the goal of the current study was to determine the feasibility of using SCE for specific genomic regions of interest by an oligonucleotide microarray followed by a NGS for targeted re-sequencing. In this pilot study, we selected *NF1* (17q11.2) as the target using 2 full-gene sequenced samples. With the existing pseudogenes, consisting of 58 exons and harboring complex genomic structures such as repetitive and Alu sequences, development of a comprehensive sequencing assay for the *NF1* is a challenge. After genomic DNA fragmentation, functional *NF1* sequences were captured and enriched by hybridizing to a custom-designed oligonucleotide microarray (385,000 features). The unbound fragments were removed through washing and the captured fragments were eluted and amplified to add specific tags for the following GS FLX sequencing. The initial call results showed 100% concordance between the SCE-NGS approach *vs*. the sequenced data. One sample possesses an insertion (an Alu repetitive element) close to exon 6 and the other sample possesses a single nucleotide deletion in exon 1. The percentage of captured and enriched pseudogene fragments was also analyzed by alignment against the reference sequence. In conclusions, the SCE-NGS approach provides advantages over the Sanger sequencing, such as avoiding initial PCR and pseudogene amplification to improve sequencing specificity, and sequencing multiple large regions of interest (including exons and introns) simultaneously. However, SCE-NGS does require high quantity and quality genomic DNA for initial fragmentation and hybridization processes that may not be suitable for certain sample types.

Genetic Basis of DYT6 Dystonia. *T. Fuchs[1], R. Saunders-Pullman[2,3], D. Raymond[2], P. de Carvalho Aguiar[4,5], A. Brashear[6], S. B. Bressman[2,3], L. J. Ozelius[1]* 1) Genetics and Genomic Sciences, Mount Sinai School of Medicine, New York, NY; 2) Department of Neurology, Beth Israel Medical Center, New York, NY; 3) Department of Neurology, Albert Einstein College of Medicine, Bronx, NY; 4) Instituto Israelita de Ensino e Pesquisa Albert Einstein, Hospital Israelita Albert Einstein, São Paulo-SP, Brazil; 5) Department of Neurology and Neurosurgery-Universidade Federal de São Paulo, São Paulo-SP, Brazil; 6) Department of Neurology, Wake Forest University School of Medicine, Winston-Salem, NC.

Dystonia is a movement disorder characterized by twisting movements and abnormal postures. The molecular pathophysiology of dystonia is not well understood, in part due to limited knowledge of the genetic basis of the disorder. At least 15 different types of dystonia can be distinguished genetically, most of which are inherited in an autosomal dominant (AD) manner with reduced penetrance. Six types, DYT1, 2, 4, 6, 7 and 13, comprise primary forms, where dystonia is the only neurologic feature. The genetic basis for only one of these, DYT1, responsible for most cases of early onset generalized dystonia, has been identified and is caused by a heterozygous three basepair in-frame deletion in the TOR1A gene. DYT6 is a primary torsion dystonia that is dominantly inherited with reduced penetrance of about 60 percent. It has been mapped to a 23cM region spanning the centromere between markers D8S2317 and D8S232 in three Amish-Mennonite families who share a disease haplotype across this region. We report here the identification of the gene causing Primary Torsion Dystonia of mixed type (DYT6). We found a founder Insertion/Deletion mutation underlying DYT6 in the Amish-Mennonite population. Furthermore, we detected several different mutations in this gene in populations of distinct ethnic origins. The discovery of the genetic basis for DYT6 affords a window into understanding the other dystonia subtypes. It furthermore provides a venue for investigation leading to deciphering the pathophysiology and devising novel treatments for this poorly understood and disabling disease.

Mitochondrial DNA content in various tissues of patients with mtDNA depletion syndrome. *L. Tang, D. Dimmock, L. Wong* Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

The mitochondrial DNA depletion syndromes (MDDS) are autosomal recessive disorders characterized by a tissue specific reduction in cellular mitochondrial DNA (mtDNA) content. However, with at least 9 genes reported to result in mitochondrial DNA depletion (ECGF-1, POLG, DGOUK, Tk2, SUCLA2, MPV17, SUCLAG1, RRM2B, and TWINKLE), mutation based testing is expensive. It would be of great value if a simple method can be used to screen for mtDNA depletion before sequencing analysis of the genes. The purpose of this study is to evaluate the potential usage of real-time quantitative polymerase chain reaction (RT qPCR) in the measurement of the mtDNA content in various tissues and to determine the relevant tissues to be used for the diagnosis of mtDNA depletion syndrome. In this study we analyzed 370 blood, 343 muscle, and 29 liver specimens to establish reference ranges for mtDNA content. The mtDNA content is increased by age in muscle but not in blood or liver samples. We also evaluated the mtDNA content in different tissues from affected patients with proven mutations in POLG, DGOUK, MPV17, and TK2 genes responsible for mtDNA depletion. As a screening test mtDNA content in blood appears to be specific but not very sensitive. Conversely, severe mtDNA depletion can be easily detected in the affected tissues such as muscle of patients with TK2, and liver of patients with MPV17, DGUOK, and POLG mutations, and could provide the basis for a rapid and accurate test.

Evidential Analysis of Genome-Wide Association Studies: Sample Size and Multiple Testing Implications. *L. J. Strug[1,2], S. E. Hodge[3,4], T. Chiang[1]* 1) Child Health Eval Sci, Hosp for Sick Children; 2) Pub Health Sci, University of Toronto, Toronto, Canada; 3) Biostat, Columbia University; 4) NYSPI, New York, NY.

Genome-wide association studies (GWAS) grapple with methodology choice for measuring strength of evidence, choosing sample size, and adjusting for multiple testing (MT). We present the evidential paradigm (EP) for GWAS analysis, which uses likelihood ratios (LRs) to measure strength of evidence (as opposed to a p-value or Bayes factor), and provides informative graphical displays for drawing inferences. We derive EP GWAS methodology (cf. Strug, Hodge 2006a,b) to (1) estimate sample size; (2) adjust for MT; and (3) provide graphical displays for analysis. (1) *Sample Size* Choose sample size to control the probability of weak association signals (W) rather than Type I error (M). Via simulation we show, for LR32 representing strong evidence, that to detect an OR1.5 at one locus with n=200 cases and n=200 controls results in W=0.134 and M=0.005. For n=300 cases and controls, W=0.039 and M=0.004. Both M and W decrease as n gets large; moreover, when W is small, M is *very* small. (2) *Multiple Testing* With M small at one locus, the Family Wise Error Rate (FWER) may still be large with N SNPs analyzed. The EP decouples error probabilities (W, M) from evidence measures and MT adjustments are performed on error rates (i.e. FWER) rather than requiring larger LRs. We present three approaches to FWER control, (a) one for a single stage design, (b) one for two-stage (replication) designs, and (c) one working with the "x-FWER," i.e., the probability of making at least x errors in N SNP tests. Method (c) chooses smaller n than (a) or (b), and (b) has smaller bounds on the FWER but higher W. (3) *Application to Cystic Fibrosis Dataset* We show GWAS plots of likelihood intervals for SNPs by base pair position. Those with LRs<32 are greyed-out, highlighting important regions. *Conclusion* EP and Frequentist likelihood-based methodologies generally provide similar results on a given set of data. However, incorporating the planning and MT adjustment approaches of the EP may identify important signals that would be interpreted as weak with Frequentist analysis.

Fast and flexible simulation of DNA sequence data. *G. K. Chen[1], P. Marjoram[1], J. D. Wall[2]* 1) Department of Preventive Medicine, Keck School of Medicine, USC, Los Angeles, CA; 2) Inst Human Genetics, Univ California, San Francisco, San Francisco, CA.

Simulation of genomic sequences under the coalescent with recombination has conventionally been impractical for regions beyond tens of megabases. Here, we present an algorithm, implemented in the program MACS, that can efficiently simulate haplotypes under any arbitrary model of population history. We present several metrics comparing the performance of MACS against several available simulation programs. Practical usage of MACS is demonstrated through a comparison of measures of linkage disequilibrium between generated program output and real genotype data from populations considered to be structured.

DNA methylation profiling in placentae of growth restricted fetuses and controls using CpG Island (CGI) microarrays. *J. Ferreira[1,2], S. Choufani[1], D. Grafodatskaya[1], C. Shuman[3], S. Keating[5], D. Chitayat[3,4], J. Kingdom[4], R. Weksberg[1,2,3]* 1) Gen & Genomics Prog, Hosp Sick Children, Toronto, ON, Can; 2) Inst Medical Sciences, Univ of Toronto, ON, Can; 3) Div Clinical and Metabolic Genetics, Hosp Sick Children, Toronto, ON, Can; 4) MFM Division, Dep Obstetrics & Gynecology, Mt Sinai Hosp, Toronto, ON, Can; 5) Dep Pathology, Mt Sinai Hosp, Toronto, ON, Can.

The purpose of this work is to validate the use of microarray technology for the detection of altered methylation profiles in fetal growth restriction (FGR) placentae in comparison with controls. DNA was extracted from placentae of 8 FGR cases and 8 controls and control blood samples. Fractioned genomic DNA and a methylated enriched fraction obtained through immunoprecipitation with an antibody to the methylated cytosine (MeDip) was co-hybridized to Agilent 244K CGI microarrays. To validate the technique we tested for reproducibility and the capability to detect known methylation differences. Pyrosequencing of bisulphite converted genomic DNA was used to validate the differences found between cases and controls after analysis of the array data. Reproducibility was confirmed by verification that 1) Correlation among placental samples and among blood samples was higher than 90%; lower correlations were found between different tissues. 2) UCSC genome browser analysis of the microarray data demonstrated the expected higher methylation levels for female versus male X chromosomes; further, known methylation differences were detected in blood versus placenta in H19 promoter and IGF2DMR2. This difference was used to model the analysis of the array data from FGR and control samples using the Partek Genomics Suite. One of the first differences found between FGR and controls has already been confirmed by pyrosequencing. Our preliminary results have shown that our methodology is reliable and can be used to compare the methylation status of CGIs between FGR and control DNA samples.

Rapid identification of mutations in the CPT1A gene using SURVEYOR nuclease. *A. B. Santani[1], T. Tischler[1], S. Narayan[1], M. Bennett[1], S. Olpin[2], C. A. Stolle[1]* 1) Dept Pathology & Lab Medicine, Children's Hospital of Philadelphia, Philadelphia, PA; 2) Sheffields Childrens Hospital, UK.

Carnitine palmitoyltransferase 1A (CPT1A) deficiency is a rare autosomal recessive disorder of mitochondrial long-chain fatty acid oxidation. CPT1 is located in the outer mitochondrial membrane and facilitates the transport of long chain fatty acids in to the mitochondria for oxidation. Mutations in the CPT1A gene (hepatic isoform) result in reduced activity of CPT I, which prevents the entry of fatty acids in to the mitochondria for energy production. CPT1A deficiency usually presents in a previously normal child as hepatic encephalopathy, precipitated by fasting or gastrointestinal illness. Typical symptoms include hypoketotic hypoglycemia and sudden onset of liver failure. CPT1A deficiency, although commonly seen in Alaskan and Hutterite populations, is quite rare in the general population with fewer than 50 cases reported worldwide. Even fewer cases have been characterized at the molecular genetics level. In this study, we developed an assay to screen for point mutations in the CPT1A gene using SURVEYOR nuclease and the Transgenomic WAVE system. Using this approach, we screened five patients with enzymatically confirmed CPT1 deficiency. Seven different mutations were identified. Of these seven variants, mutations in two residues have been previously reported. The remaining five were novel mutations that included three nonsense mutations, one splice site mutation and a 1469 bp deletion encompassing exon 12 of the CPT1A gene. All five patients were successfully genotyped using this assay, in that, these patients were either homozygotes or compound heterozygotes for mutations in the CPT1A gene i.e., a 100% detection rate in enzymatically confirmed CPT1A deficient patients. The SURVEYOR nuclease assay is a highly sensitive technique for rapid detection of mutations at a lower cost compared to direct sequencing. Use of genetic testing for early identification of CPT1A in patients and at risk family members improves diagnostic certainty and reduces morbidity since early recognition of clinical manifestations will allow timely intervention and improved outcome.

Functional impact of the expression of the transcription repressor ETV6 in humanized mice models of leukemia.

J. Larose[1], M. Bossolasco[1], S. Langlois[1], F. Fontaine[1], S. Desjardins[1], N. Heveker[1,3], E. Haddad[1,4], D. Sinnett[1,2,3] 1) Hematology-Oncology Dept, Research center CHU Sainte-Justine, Montreal, Qc, Canada; 2) Pediatric Dept, University of Montreal, Montreal, Qc, Canada; 3) Biochemistry Dept, University of Montreal, Montreal, Qc, Canada; 4) Microbiology and immunology Dept, University of Montreal, Qc, Canada.

Deletions at chromosome 12p12-13 are observed in 26 to 47% of childhood pre-B acute lymphoblastic leukemia (ALL) cases suggesting the presence of a tumour suppressor gene (TSG). Accumulating genetic and functional evidence points to ETV6 as being the most probable TSG targeted by the deletions. ETV6 is a ubiquitously expressed transcription factor of the ETS family with very few known targets. To better understand the contribution of ETV6 to leukemia, we have developed humanized mouse models of leukemia. This was achieved by injecting pre-B ALL cell lines Nalm-6 (ETV6+) and REH (ETV6-) in very immunodeficient mice NOD/SCID/c^{null}. In both models, we were able to recapitulate the human leukemia features but with different organ dissemination patterns: Nalm6 cells were found mainly in circulation (peripheral blood and spleen) whereas REH cells were found in bone marrow. To determine to which extend ETV6 status was responsible for differences in colonization capabilities, we genetically modified using lentiviral constructs both cell lines by restoring (overexpression) or silencing (shRNA) ETV6 expression. The modulation of ETV6 expression had a substantial impact on the dissemination pattern suggesting a role in either homing or migration processes. Both *in vitro* and *in vivo* studies indicate that ETV6 contributes to cellular migration. This work identify ETV6 as a key player in leukemia cells migration and provide an *in vivo* model for further studies on the role of ETV6 in human leukemogenesis.

Using Adaptive Numerical Integration for Multidimensional Genetic Problems. *S. Seok[1], Y. Huang[1], M. Evans[2], V. Vieland[1]* 1) Battelle Center for Mathematical Medecine, The Research Institute at Nationwide Childrens Hospital, Columbus, OH; 2) Dept. of Statistics, University of Toronto, Toronto, Canada.

Various statistical methods in genetics require numerical integration (NI). While MCMC is frequently used for this purpose, MCMC is not feasible in some settings. For example, the PPL framework requires integration of multidimensional likelihoods lacking closed form solutions, and for which the design of suitable Gibbs or Metropolis-Hastings samplers does not seem possible. We have therefore developed an alternative approach to NI for this problem which may be useful in other contexts as well. This approach is based on an integration algorithm known as DCUHRE. DCUHRE is a sub-region adaptive algorithm using an embedded family of fully symmetric multiple quadrature rules. These rules have the property that they integrate polynomials, up to a certain degree based on the number of points in the rule, exactly over hypercubes. More significantly, DCUHRE adapts to the integrand by iteratively subdividing the domain of integration into subregions based upon error estimates of the integral approximations in current subregions. This results in evaluating the integrand more frequently at points where significant contributions to the integral occur. Further DCUHRE records meaningful error estimates. We have implemented a version of DCUHRE specifically adapted to genetic likelihoods in the PPL software package Kelvin. This includes, for instance, ordering constraints on penetrances via variable transformation to treat the penetrance region as a hypercube. We have performed extensive comparisons against a brute force fixed-grid approach to NI as an available method to approximate these integrals with arbitrarily high precision. We show that our algorithm is highly accurate and efficient when applied to genome-wide gene mapping studies. In particular, it requires less than 0.01% of the brute force calculations for corresponding accuracy. In practical terms, this allows us to complete in 60 hours a genome-wide linkage or linkage disequilbrium scan that previously could have taken 7 years using the brute force approach.

Identification and functional characterization of sequence variation in the Zellweger spectrum of peroxisome biogenesis disorders. *W. Yik[1], S. Steinberg[2], K. Ramaswamy[1], A. Moser[2], H. Moser[2], J. Hacia[1]* 1) Dept Biochem & Molecular Biol, Inst Genetic Medicine, Los Angeles, CA; 2) 2Peroxisomal Diseases Laboratory Kennedy Krieger Institute Baltimore, MD, USA.

Peroxisome biogenesis disorders (PBD) are a heterogeneous group of autosomal recessive neurodegenerative disorders affecting multiple organ systems. Approximately 80% of PBD patients are classified in the Zellweger syndrome spectrum (PBD-ZSS). We conducted sequencing assays to survey the mutational spectrum in the coding regions and adjacent splice junction of five PEX genes (PEX1, PEX6, PEX10, PEX12, and PEX26) critical for normal peroxisome assembly in a cohort of 58 PBD-ZSS patients. We identified 69 unique sequence variants, including 18 novel mutations predicted to disrupt protein function and 2 novel silent variants. For two patients where two different PEX genes contained missense alleles affecting conserved amino acids, we conducted cell fusion complementation analyses to assign the identity of the disease-causing gene. By combining genotype from these 58 individuals with an additional 33 other PBD-ZSS patients, we found that mutations in the PEX1 (59.3%), PEX6 (9.9%), PEX10 (3.3%), PEX12 (7.7%), and PEX26 (5.5%) genes account for 85.7% of PBD-ZSS patients. Overall, we provide empirical data to estimate the relative fraction of disease-causing alleles that occur in the coding and splice junction sequences of these five PEX genes as well as identifying instances where mutations are found in multiple PEX genes. This is beneficial for efforts aimed at establishing rapid, sensitive, and cost-effective clinical diagnostics for PBD-ZSS patients.

Frequency distribution and selection in 4 pigmentation genes in Europe. *M. P. Donnelly, W. C. Speed, J. R. Kidd, A. J. Pakstis, K. K. Kidd* Dept Genetics, Yale Univ Sch Med.

Pigmentation is one of the more obvious forms of variation in humans, particularly in Europeans where one sees more within group variation in hair and eye pigmentation than in the rest of the world. We studied 4 genes (SLC24A5, SLC45A2, OCA2 and MC1R) that are believed to contribute to the pigment phenotypes in Europeans. SLC24A5 has a single functional variant that leads to lighter skin pigmentation. Data on 83 populations worldwide (including 55 from our lab) show the variant (at rs1426654) has almost reached fixation in Europe, Southwest Asia, and North Africa, has moderate to high frequencies (.2-.9) throughout Central Asia, and has frequencies of .1-.3 in East and South Africa. The variant is essentially absent elsewhere. SLC45A2 also has a single functional variant (at rs16891982) associated with light skin pigmentation in Europe. Data on 84 populations worldwide show the light skin allele is nearly fixed in Northern Europe but has lower frequencies in Southern Europe, the Middle East and Northern Africa. In Central Asia the frequency of the SLC45A2 variant declines more quickly than the SLC24A5 variant. It is absent in both East and South Africa. In OCA2 we typed 4 SNPs (rs4778138, rs4778241, rs7495174, rs12913832) with a haplotype associated with blue eyes in Europeans. This haplotype shows a Southeastern to Northwestern pattern in Europe with frequencies of .25 (.05 homozygous) in the Adygei to .85 (.75 homozygous) in the Danes. In MC1R we typed 5 SNPs (rs3212345, rs3212357, rs3212363, C_25958294_10, rs7191944) that cover the entire MC1R gene and found a predominantly European haplotype that ranges in frequency from .35 to .65 in Europe, reaching its highest levels in Southwest Asia and Northwestern Europe. Extended Haplotype Heterozygosity (EHH) and normalized Haplosimilarity (nHS) show evidence of selection at SLC24A5 in not only our European and Southwest Asian populations but also our East African populations. Neither SLC45A2 or OCA2 showed evidence of selection in either test. MC1R did not show evidence of selection for our European specific haplotype but we did see some evidence both upstream and downstream in our nHS test in Europe.

Identifying Recurrent DNA Copy Number Aberrations in Lung Cancer by Correlation Matrix Diagonal Segmentation. *Q. Zhang[1], L. Ding[2], A. Kraja[1], I. Borecki[1], M. A. Province[1]* 1) Division of Statistical Genomics, Washington University School of Medicine, St. Louis, MO; 2) Genome Center, Washington University School of Medicine, St. Louis, MO.

As a phenomenon of DNA amplification or deletion taking place at the same chromosomal region across multiple cancer patients, recurrent copy number aberration (RCNA) may play an important role in molecular mechanism of oncogenesis and provide useful information for the diagnosis and treatment of cancers. Most existing approaches of RCNA analysis require data discretization for individual samples, which may reduce statistical power and suffer computational burden. We have developed a novel approach, Correlation Matrix Diagonal Segmentation (CMDS), which directly uses raw copy number data to identify RCNAs with no need of discretization for individual samples. CMDS significantly reduces computational time, and therefore provides an efficient tool for genome-wide and large-population-based analysis. Simulation shows that CMDS can achieve higher statistical power compared with typical discretization-based approaches. Applying CMDS to the Affymetrix Human Mapping 250K Sty SNP Array data of matched tumor and normal tissue samples from 357 lung cancer (adenocarcinoma) patients in the Tumor Sequencing Project (TSP), we identify multiple RCNA regions on chromosomes 1,5,6,7,8,9,11,12,14,17,19 and 22. We investigate the distribution of these RCNAs among individual samples and identify five regions (5q35, 7p12, 11q13, 12p12, 12q15) showing significant differences of amplification frequencies between ever-smoking and never-smoking patients. Multiple tumor-related genes (EGFR, KRAS, CCND1, MDM1 and MDM2, etc.) are located within these regions. Of particular interest are the nuclear phosphoproteins MDM1 and MDM2, which bind to and inhibit trans-activation by tumor protein TP53. The stronger amplifications of the two genes in never-smoking patients are validated using qPCR data from an independent cohort of 121 patients. MDM2 is also validated at the RNA expression level by the data from the Affymetrix HGU133 arrays of 75 patients.

Functional variants in *TGFB1* are associated with increased airway responsiveness and disease exacerbations in children with asthma. *S. Sharma[1,2], B. A. Raby[1,2,3], M. Soto-Quirós[4], L. Avila[4], A. J. Murphy[1,5], B. Klanderman[1], J. Sylvia[1], S. T. Weiss[1,2,3], J. C. Celedón[1,2,3]* 1) Dept Respiratory Epidemiology, Channing Laboratory, Boston, MA; 2) Division of Pulmonary and Critical Care Medicine, Center for Genomic Medicine, Department of Medicine, Brigham and Women's Hospital, Boston, MA; 3) Harvard Medical School, Boston, MA, United States; 4) Division of Pediatric Pulmonology, Hospital Nacional de Niños, San José, Costa Rica; 5) Department of Biostatistics, Harvard School of Public Health, Boston, MA.

Rationale: There has been no association study of TGFB1 and airway responsiveness or disease exacerbations in asthmatics. **Objectives**: To test for association between single nucleotide polymorphisms (SNPs) in TGFB1 and asthma severity in childhood. **Methods**: We tested for association between 9 SNPs in TGFB1 and asthma and indicators of severity (airway responsiveness and exacerbations) in two cohorts: 416 Costa Rican parent-child trios, and 465 families of white children in the Childhood Asthma Management Program (CAMP). We also tested for interaction between these SNPs and dust mite allergen on asthma severity. The analysis was conducted with the family-based association test statistic under a dominant genetic model. **Results**: The A allele of the promoter SNP rs2241712 was associated with increased airways responsiveness in Costa Rica (P=0.0006) and CAMP (P=0.01), and the C allele of a functional SNP (rs1800469) was associated with increased airways responsiveness in both cohorts (P 0.02). There was an interaction between the C allele of SNP rs1800471 and dust mite allergen exposure on airways responsiveness (P=0.03 in both cohorts). The T allele of a coding SNP in TGFB1 (rs1982073) was associated with a reduced risk of asthma hospitalizations (P=0.01 in both samples). There was also an interaction between the A allele of SNP rs2241712 and dust mite allergen on asthma-related hospitalizations in both cohorts. **Conclusions**: SNPs in TGFB1 may influence airway responsiveness and disease exacerbations in children with asthma. Dust mite allergen exposure may modify the effect of TGFB1 SNPs on airways responsiveness and hospitalizations in children with asthma.

Regulation of the master transcription factor RUNX2 by the nuclear. *P. Fonseca[1], T. Yang[1,2], T. Bertin[1], B. Dawson[1,2], Y. Chen[1], G. Zhou[3], B. Lee[1,2]* 1) Baylor Col Medicine, Houston, TX; 2) HHMI; 3) Case Western Reserve University.

RUNX2 is a member of the RUNT family of transcription factors that is essential for osteoblast formation and chondrocyte maturation. Loss-of-function mutations result in cleidocranial dysplasia (CCD). RIP (RUNX2-Interacting Protein) was isolated in a yeast 2-hybrid screen of a human osteosarcoma cDNA library. Two families with rearrangements involving the upstream region of the gene have a CCD phenotype with upregulation of RIP. RIP down-regulated the transactivation by RUNX2 in a dose-dependent manner in 10T1/2 and ROS17/2 cells. It localizes to the inner nuclear membrane. Its major components are lamins, which have an important role in nuclear organization and transcriptional regulation. Mutations in lamin and associated proteins result in laminopathies, including mandibuloacral dysplasia (MAD) that partially phenocopies CCD. RUNX2 function can be regulated at different levels, but its subnuclear localization is crucial. We hypothesize that the skeletal defects observed in laminopathies are due to the disruption of lamin interactions with key transcriptional regulator RUNX2. Knockdown of Rip in ROS17/2 cells up-regulates Runx2, Alp and Ocn while silencing Lmna down-regulates both Runx2, Osx and downstream targets. Lamin interacts with RIP through the tail domain, which is mutated in MAD patients. Lamin versions carrying MAD mutations have altered binding properties but show similar nuclear localization. RUNX2 partially co-localizes at the nuclear rim in ROS17/2 cells and mouse calvarial osteoblasts. Lamin is required for terminal differentiation of C2C12 cells into osteoblasts. To evaluate the in vivo consequences of RIP gain-of-function, we generated transgenic mice over-expressing RIP in the osteoblast. Multiple lines exhibited severe osteopenia and mineralization defects. Bone formation and mineral apposition rates were markedly reduced. We also observed a higher number of adipocytes in the bone marrow. Altogether, these are signs of a premature ageing bone. These data link RUNX2 function, the novel RIP protein, and lamin in a transcriptional regulatory complex and suggest a new mechanism for regulating skeletogenesis at the nuclear lamina.

The CHD7 protein, mutated in CHARGE syndrome, binds to enhancers marked with mono- and di-methylated H3K4. *M. P. Schnetz, C. F. Bartels, D. Balasubramanian, R. Balaji, T. LaFramboise, P. C. Scacheri* Genetics, Case Western Reserve University, Cleveland, OH.

CHARGE syndrome is characterized by multiple birth defects including coloboma of the eye, heart defects, choanal atresia, growth retardation, genital and ear abnormalities. De novo mutations in the gene encoding chromodomain helicase DNA-binding protein 7 cause most cases of CHARGE syndrome, but little is known about the function of the protein. To investigate the potential role of CHD7 in transcription and CHARGE syndrome, we mapped the distribution of the CHD7 protein on chromatin using the approach of ChIP on tiled microarrays (ChIP-chip). These studies were performed in human colorectal carcinoma cells, human neuroblastoma cells, and mouse embryonic stem (ES) cells before and after differentiation into neural precursor cells. The results indicate that CHD7 localizes to discrete locations along chromatin that are specific to each cell type. The CHD7 sites demonstrate several features of gene enhancers. First, most of CHD7 binding occurs >2 kb from 5' ends of genes, which is consistent with the location of enhancers relative to their target genes. Second, most of the CHD7 sites are contained within DNase hypersensitive sites that are known to harbor regulatory elements such as enhancers. Third, genes located within the vicinity of the CHD7 sites are expressed at relatively high levels. Fourth, a significant number of the CHD7 sites overlap with enhancers that were independently identified by ChIP-chip and computationally based methods. We also determined that the cell-specific binding of CHD7 correlates with a subset of histone H3 mono- and di-methylated at lysine 4 (H3K4me1/2). The CHD7 sites change concomitantly with H3K4me1/2 during ES cell differentiation, suggesting that H3K4me1/2 is part of the epigenetic signature that defines lineage-specific recruitment of CHD7 to enhancers. These findings suggest that CHD7 influences the rate of transcription by binding to gene enhancers, raising the possibility that the congenital anomalies in CHARGE syndrome are due to alterations in transcription of tissue-specific genes normally regulated by CHD7 during development.

Ancestry Informative Marker Sets for Determining Continental Origin: Validation and extension using Human Genome Diversity Panels. *R. Nassir[1], R. Kosoy[1], C. Tian[1], P. A. White[2], L. M. Butler[1], G. Silva[3], R. Kittles[4], M. E. Alarcon-Riquelme[5], P. K. Gregersen[6], J. W. Belmont[7], F. M. De La Vega[2], M. F. Seldin[1]* 1) Rowe Program Gen, Univ California, Davis, Davis, CA; 2) Applied Biosystems, Foster City, CA; 3) Obras Sociales del Hermano Pedro, Antigua, Guatemala; 4) Univ Chicago, Chicago IL; 5) Uppsala Univ, Uppsala, Sweden; 6) North Shore-LIJ Res Inst, Manhasset, NY; 7) Baylor College Medicine, Houston, TX.

To provide a resource for assessing continental ancestry in a wide variety of human genetic studies we identified, validated and characterized a set of 128 ancestry informative markers (AIMs). The markers were chosen for informativeness, genome-wide distribution, and genotype reproducibility on two platforms (TaqMan and Illumina arrays). Initial studies examined genotyping data from 825 subjects with diverse ancestry, including European, East Asian, Amerindian, West African, South Asian, Mexican, and Puerto Rican. A comprehensive set of 128 AIMs and subsets as small as 24 AIMs were shown to be useful for ascertaining the origin of subjects from particular continents, and to correct for population stratification in admixed population sample sets. Additional analyses using Human Diversity Panel Genotypes have confirmed the ability of the 128 AIMs to distinguish diverse population groups including those not represented in our previous data sets. These included populations from Oceana, and multiple additional South Asian, East Asian, Sub-Saharan African, Amerindian and European populations. In addition to being able to effectively identify Oceanic groups, these AIMs provide some population substructure information, for example, distinguishing Middle Eastern from Northern European population groups and Pygmi from other Sub-Saharan African population groups. Our findings provide general guidelines for the application of specific AIM subsets as a resource for wide application in subject set selection and in association testing. We conclude that investigators can use TaqMan assays for the selected AIMs as a simple and cost efficient tool to control for large differences in continental ancestry when conducting association tests in ethnically diverse populations.

A hotspot splice site mutation in LEPRE1 affects splice form, responsible for prolyl 3-hydroxylation of type I collagen, and causes recessive osteogenesis imperfecta. *P. J. Coucke[1], A. Willaert[1], S. Symoens[1], K. Gevaert[2,3], H. Kayserili[4], A. Megarbane[5], F. Malfait[1], A. De Paepe[1]* 1) Dept Med Genet, Univ Hosp Ghent, Ghent, Belgium; 2) Dept of Bioch, Ghent Univ, Belgium; 3) Dept of Medical Protein Res, VIB-Ghent, Belgium; 4) Div of Medical Genet, Instanbul Univ, Turkey; 5) Unit of Med Genet, University St-Joseph, Lebanon.

Recently, it has been shown that recessive forms of Osteogenesis Imperfecta (OI) can be caused by the absence of 3-hydroxylation of Pro986 in the 1(I)-collagen chain, resulting from defects in prolyl 3-hydroxylase 1 (P3H1) or cartilage-associated protein (CRTAP). Together with cyclophilin B (CyPB), P3H1 and CRTAP are constituents of the prolyl 3-hydroxylation complex. We screened LEPRE1 (encoding P3H1), CRTAP and PPIB (encoding CyPB) in a European/Middle Eastern cohort of lethal/severe OI-like patients without a type I collagen mutation. While no mutations were found in CRTAP and PPIB, 4 novel homozygous and compound heterozygous mutations were identified in LEPRE1 in 4 probands. Surviving probands showed a severe short stature, a relatively round face, white sclerae and rhizomelia with long hypermobile hands. Radiographs revealed progressively increasing severe matrix disorganisation with bulbous metaphyses. Interestingly, a hotspot splice site mutation was identified in two of four probands. As confirmed by RT-PCR, the latter mutation converts a suboptimal into an optimal donor splice site in intron 14, resulting in a full switch in donor splice site utilization. Consequently, this mutation affects only one of the three LEPRE1 mRNA splice forms, detected in this study. The affected splice form encodes a 736 amino acid protein. While western blotting and immunocytochemical analysis of fibroblast cultures showed absence of P3H1 protein in patients compared to controls, mass spectrometry and SDS-urea-PAGE data indicated respectively a severe reduction of 1(I)Pro986 3-hydroxylation and a resulting overmodification of type I (pro)collagen chains in the patients. These analyses showed that the 3-hydroxylation function of P3H1 is restricted to a single protein splice form, comprising 736 amino acids.

The association of lobular breast cancer with germline mutations of *CDH1* . *K. Schrader[1,2], S. Masciari[3], N. Boyd[2], J. Senz[1], P. Kaurah[2], M. B. Terry[4], E. John[4], I. L. Andrulis[4], J. Knight[4], F. P. O'Malley[4], M. Daly[4], P. Bender[4], M. C. Southey[4,5], J. L. Hopper[4,5], J. Garber[3], D. G. Huntsman[1,2], kConFab* 1) Department of Pathology and Laboratory Medicine, UBC, Vancouver, BC; 2) Hereditary Cancer Program, BCCA, Vancouver, BC; 3) Department of Medical Oncology, DFCI, Boston, MA; 4) Breast Cancer Family Registry (B-CFR); 5) University of Melbourne, Melbourne, Vic.

Background: *CDH1* encodes the cell-cell adhesion molecule, E-cadherin, for which loss of expression facilitates the infiltrative and metastatic potential of cancers. Germline mutations in *CDH1* are associated with hereditary diffuse gastric cancer (HDGC), and in this setting female carriers have been estimated to have a 39-50% risk of lobular breast cancer (LBC) by age 80 years.

Aim: To determine the frequency of *CDH1* germline mutations in individuals with early-onset LBC or those with LBC and a family history of multiple breast cancers but no gastric cancers.

Methods: Germline DNA analysis of *CDH1* in women with LBC, for whom germline *BRCA1* and *BRCA2* mutations have been excluded, who have been (1) diagnosed before the age of 45 years or (2) diagnosed at any age and have a family history of breast cancer.

Results: Analysis of 194 LBC cases has thus far revealed two novel missense mutations predicted to affect protein function. Functional assays to assess their pathogenicity along with germline analyses of the remaining 200 cases are currently underway. Several unreported silent changes have also been identified and will be measured in a case-control sample to assess whether they are associated with LBC risk.

Conclusion: Germline *CDH1* mutations may cause a small proportion of familial and early onset LBC.

Multi-study fine mapping of a chronic obstructive pulmonary disease susceptibility locus on chromosome 2q. *C. P. Hersh[1], S. G. Pillai[2], G. Zhu[2], D. A. Lomas[3], I. C. G. N. Investigators[4], P. Bakke[5], A. Gulsvik[5], D. L. DeMeo[1], A. A. Litonjua[1], J. J. Reilly[1], E. K. Silverman[1]* 1) Brigham and Women's Hospital, Boston, MA; 2) GlaxoSmithKline, Research Triangle Park, NC; 3) Cambridge Institute for Medical Research, Cambridge, UK; 4) International COPD Genetics Network; 5) University of Bergen, Norway.

Background: Two independent family-based studies of chronic obstructive pulmonary disease (COPD) have identified linkage for COPD-related traits to chromosome 2q. We hypothesized that merging results of high-resolution SNP mapping in four separate populations would lead to the identification of COPD susceptibility genes.

Methods: Within the chromosome 2q linkage region, 2843 SNPs were genotyped in 1839 individuals from 603 families from the International COPD Genetics Network (ICGN) and in 806 COPD cases and 779 controls from Norway. 2484 SNPs were genotyped in 309 patients with severe COPD from the National Emphysema Treatment Trial (NETT) and 330 community controls. 149 SNPs were genotyped in 949 individuals from 127 families in the Boston Early-Onset COPD Study. Family-based and case-control genetic association tests were performed, as appropriate for each study population.

Results: Merging the results of the two case-control analyses, 15 of the 790 overlapping SNPs had a combined p0.01. Five of these 15 SNPs were associated with COPD-related traits in the ICGN families. The association with one of these five SNPs, located in the gene XRCC5, was replicated in the Boston Early-Onset COPD Study families, with a combined $p = 2 \times 10^{-5}$ across the four studies. Genotype imputation was used to confirm the association with XRCC5.

Conclusions: By combining data from COPD genetic association studies conducted in four independent patient samples, we have identified XRCC5, an ATP-dependent DNA helicase required for immunoglobulin V(D)J rearrangement, as a potential COPD susceptibility gene.

A new case of keratin 14 functional knockout causing recessive EBS. *A. Décha[1], M. Titeux[1], L. Tonasso[1], C. Prost-Squarcioni[2], V. Pendaries[1], G. Albérola[1], J. E. Mejía[1], A. Hovnanian[1,3]* 1) U563, Inserm, Toulouse, France; 2) 2EA3410, Histology Laboratory, University of Paris XIII, Bobigny, France; 3) Purpan Hospital, Departement of Medical Genetics, Toulouse.

Epidermolysis bullosa simplex (EBS) is a group of inherited skin disorders characterized by intra-epidermal blistering upon mild trauma. Most cases are caused by dominant missense mutations in either the *KRT5* or *KRT14* genes encoding keratins 5 and 14, respectively. Only 5% of EBS cases are inherited recessively. We describe a 30-year-old patient with recessive EBS Köbner, born to first-cousin parents. He presented with generalized skin blistering, often hemorrhagic and more prominent on the hands and feet, associated with hyperkeratosis and the absence of dermatoglyphs. Mucosal involvement was observed as blisters on the tongue and an anal fissure. He had a hoarse voice since birth and developed bilateral hypoacousia since age 10. Electron microscopy of the skin revealed a cleavage within the cytoplasm of basal keratinocytes, and the absence of keratin filaments. Keratin 5 immunostaining in skin sections was normal, while keratin 14 was absent and keratin 15 slightly reduced. *KRT14* sequencing on leukocyte DNA showed a homozygous 1-bp deletion (c.827delC) in exon 4 resulting in a frameshift and a premature termination codon (p.Pro276LeufsX4). Western-blot analyses demonstrated the complete lack of keratin 14, while keratin 5 was normally expressed. Quantitative RT-PCR analysis on keratinocyte primocultures confirmed nonsense-mediated *KRT14* mRNA decay while the *KRT5* and *KRT15* mRNA levels were normal. Confocal microscopy demonstrated the presence of keratin 5/keratin 15 heteropolymers in both normal and EBS cultured keratinocytes, suggesting that keratin 15 could partially compensate for the loss of keratin 14, as previously described in keratin 14 knock-out mice and in patients affected with recessive EBS. The parents and the offspring of the patient, who are heterozygous carriers, will be carefully examined for clinical features reminiscent of Naegeli-Franceschetti-Jadassohn syndrome (NFJS), a dominant disorder proposed to arise through keratin 14 haploinsufficiency.

Association between LOXL1 polymorphisms and early-onset primary open-angle glaucoma using ordered subset case-control association analysis (OSACC). *K. Crooks[1], X. Qin[1], Y. Liu[1], J. Gibson[1], K. Hutchins[1], P. Challa[2], R. Allingham[1,2], M. Hauser[1,2], S. Schmidt[1]* 1) Center for Human Genetics, Duke University, Durham, NC; 2) Department of Ophthalmology, Duke University Eye Center, Duke University Medical Center, Durham, NC.

Glaucoma is a complex genetic disorder and a leading cause of blindness for which there is no cure. One useful tool that may ultimately lead to novel therapeutic approaches is the identification of DNA sequence variants that modulate glaucoma risk. Recently, it has been reported that polymorphisms in the lysyl oxidase-like 1 (LOXL1) gene are associated with pseudoexfoliation glaucoma (XFG) in several distinct populations. Our group found no association between 14 tagging SNPs in LOXL1 and the most common form of glaucoma, primary open angle glaucoma (POAG), using 280 cases and 224 controls. However, since POAG is a genetically heterogeneous disease, we applied a novel algorithm for ordered subset case-control association analysis, using age at diagnosis as the clinical covariate. We found that three intronic LOXL1 SNPs showed a significantly stronger association with POAG risk in the subset of cases diagnosed at or before 55 years of age (n=100; permutation test p-values 0.02-0.04). Interestingly, for each of these SNPs, the allele that confers an increased risk of XFG (OR 2.0-2.3 per allele, additive model) is protective for early-onset POAG (OR 0.61-0.63 per allele). For example, allele G at rs4337252 has a frequency of 68.4%, 50.7% and 38.1% in XFG cases, normal controls and early-onset POAG cases, respectively (p=0.001 for XFG; p=0.005 for early-onset POAG). Our results extend the potential role of LOXL1 variants in modulating glaucoma susceptibility, although the alleles that confer risk for XFG appear to be protective for early-onset POAG. Given this observation, we hypothesize that a different biological mechanism than the non-synonymous coding changes implicated in XFG explains the decreased risk of POAG tagged by these intronic sequence variants of currently unknown function. Our data confirm that age-at-diagnosis is an important clinical variable for dissecting the genetic heterogeneity of POAG.

A demogenetic analysis of the LDLR-W66G mutation in the Saguenay-Lac-Saint-Jean (Quebec, Canada) population. *M. Tremblay[1], A. Achkar[1], D. Gaudet[2], D. Brisson[2]* 1) BALSAC Project, Univ Quebec, Chicoutimi, PQ, Canada; 2) Montreal University Community Genomic Medicine Center, Chicoutimi, Qc, Canada.

Familial hypercholesterolemia (FH) is the most prevalent monogenic disorder worldwide. FH is characterized by high plasma concentrations of low-density lipoprotein (LDL)-cholesterol and apolipoprotein B, tendinous xanthomas and an increased risk of premature coronary artery disease. Most often FH is caused by mutations in the LDL receptor (LDLR) gene. FH affects approximately 1 per 350 individuals among Caucasians, but the prevalence is significantly higher in some populations. In the Saguenay-Lac-St-Jean (SLSJ) region (Quebec, Canada), the prevalence of FH is 1:83 and almost 90% of all cases are the consequence of two LDLR gene mutations: a >15 kb deletion in the promoter and exon 1 and a missense mutation (W66G) in exon 3. This study aimed to identify the demogenetic factors that can explain the frequency and the distribution of the LDLR-W66G mutation within the SLSJ population. Analysis was performed on a sample of extended genealogies from 64 FH subjects carrying the LDLR-W66G mutation and 64 non-FH controls. The genealogies were reconstructed using data from the BALSAC population register. Number and occurrences of ancestors, mean genealogical depth, origins and genetic contribution of founders, and kinship coefficients between subjects and controls were measured using the S-Plus based GENLIB software package. Results show that on average, the genealogies have a depth of 10 generations, with some branches reaching up to 16 generations. These genealogies contain more than 300000 occurrences of ancestors, with an average of 16 (controls) to 20 (subjects) occurrences per ancestor. Kinship coefficients are significantly higher among subjects, where they reach a mean value of 0.009 at the 12th generation. Results also indicate that the founders who contributed most to the subjects gene pool came from France some 350 years ago. **Funding**: Supported by the ECOGENE-21 project (CIHR TEAM grant # CTP-82941) and by the Social Sciences and Humanities Research Council of Canada (grant # 410-2006-1414).

Preimplantation Genetic Diagnosis (PGD) for Metaphyseal Chondrodysplasia, Schmid Type [COL10A1, TYR628TER]and Aneuploidy. *E. Pomerantseva, I. Barsky, T. Sharapova, S. Rechitsky, Y. Verlinsky* Reproductive Genetics Institute, Chicago, IL.

Schmid metaphyseal chondrodysplasia (SMC) is a dominantly inherited disorder of the osseous skeleton, characterized by a short stature, coxa vara and a waddling gait. SMC is determined by mutations in the type X collagen gene, COL10A1, which is a short chain collagen expressed in hypertrophic chondrocytes during bone growth. Nonsense mutation (Y628X) in COL10A1 gene was identified in a sporadic case of Schmid metaphyseal hondrodysplasia (McIntosh et al. 1995). We performed PGD for a patient with such a *de novo* mutation, using nested and hemi-nested multiplex PCR protocol for the mutation and tightly linked short tandem repeats (STRs). Normal and mutant haplotypes were established by single sperm analysis, and the mutation analysis was performed simultaneously with aneuploidy testing as described earlier (Rechitsky et al. 2006). The final protocol included D6S1603, D6S401, 6S454, D6S1706, D6S304 markers along with mutation, and aneuploidy markers for 13, 18, 21, X and Y chromosomes. Mutation was studied by restriction endonuclease digestion and electrophoresis, while polymorphic markers were analyzed by fluorescent fragment analysis. Single blastomeres from 9 embryos were tested, of which 4 were found to be unaffected and normal for chromosomes 13, 18, 21, X and Y, two inherited paternal mutation and another two were found aneuploid. As no data were obtained for one of the embryos, a blastocyst biopsy was performed for this embryo on day 5, showing the normal genotype. Two out of five normal embryos were transferred, resulting in singleton pregnancy and delivery of a healthy mutation free child.

Dissecting the genetic architecture of type 1 diabetes via genome-wide association of 16,783 individuals. *J. C. Barrett[1], B. Akolkar[2], P. Concannon[3], H. A. Erlich[4], C. Julier[5], G. Morahan[6], J. Nerup[7], C. Nierras[8], F. Pociot[9], J. A. Todd[1], N. M. Walker[1], J. D. Cooper[1], D. J. Smyth[1], H. Schuilenburg[1], V. Plagnol[1], J. Allen[1], S. S. Rich[10], D. G. Clayton[1], The Type 1 Diabetes Genetics Consortium* 1) CIMR, Cambridge, UK; 2) NIDDK, NIH; 3) CPHG, University of Virginia; 4) Roche Molecular Systems; 5) Institut Pasteur, Paris, France; 6) WAIMR, Perth, Australia; 7) Steno Diabetes Center, Denmark; 8) Juvenile Diabetes Research Fund; 9) Steno Diabetes Center, Denmark; 10) Wake Forest University School of Medicine.

Identification of loci that contribute to risk of Type 1 Diabetes (T1D),has accelerated in the last few years with the advent of genome-wide association studies (GWAS). To date, nine non-HLA loci have been established; however, these loci do not fully explain the genetic risk of T1D. Therefore, the T1DGC has undertaken a new GWAS with the intent of jointly analyzing newly generated and pre-existing data to obtain maximum power to detect new T1D risk loci. The combined study comprises a meta-analysis of three distinct scans: 1963 cases/3326 controls (Wellcome Trust Case Control Consortium, Affymetrix 500K), 1785 cases/1727 controls (GoKinD and NIMH, Affymetrix 500K), which have been analysed previously, and new data from 3983 cases/3999 controls (T1DGC, Illumina HumanHap 550K). We have used a subset of 1500 controls genotyped on both platforms to impute genotypes in each sample for SNPs on the other platform. The combined dataset, which features 7731 cases and 9052 controls with imputation or direct genotype data at nearly 850,000 SNPs, is the largest ever assembled for a binary disease trait. The initial meta-analysis overwhelmingly confirms (p 10^{-9}) all nine previously confirmed non-HLA associations (as well as the MHC) and provides intriguing evidence of association at a number of other candidate loci. In addition, the meta-analysis reveals 14 previously unsuspected loci with p 10^{-7}, 27 with p 10^{-6}, and over 100 with p 10^{-5}. These results, as well as replication genotyping for the top tier of hits in an independent and similarly sized panel will be presented.

Joint maximum likelihood estimation of relatedness and allele frequencies. *A. Anderson* Dept Mathematics, Western Washington Univ, Bellingham, WA.

We present a method for estimating relatedness parameters (e.g. probabilities of sharing 0, 1, or 2 alleles IBD, kinship coefficients, etc.) and allele frequencies from a set of individuals with unknown pedigree. Our approach is iterative: We begin with intial guesses for the allele frequencies, then alternate between using these allele frequencies to find maximum likelihood estimates for the relatedness parameters and using our estimated kinship coefficients to update our allele frequency estimates. The method is tested on family data from the CEPH database and found to work fairly well.

Selecting SNPs to Correctly Predict Ethnicity. *J. N. Sampson[1], K. K. Kidd[2], J. R. Kidd[2], H. Zhao[1]* 1) Dept of Biostatistics, Yale University, New Haven, CT; 2) Dept. of Genetics, Yale University School of Medicine, New Haven, CT.

Because allele frequencies at many SNPs differ across populations, genotyping appropriate SNPs can offer insight into an individual's ancestry or ethnicity. For many forensic applications, it would be desirable to select a small group of highly informative SNPs, such that by genotyping only those SNPs, we could accurately predict the ethnicity for nearly any person. With this goal in mind, we preselected a group of 249 candidate SNPs, most already identified to have high Fst values in a small number of populations (e.g. HapMap), and then genotyped these candidate SNPs in approximately 2500 subjects from more than 40 populations. Here, we will discuss our novel method of selecting a small subgroup of these SNPs that can accurately distinguish populations. Specifically, we use a greedy algorithm to find subsets of SNPs that minimize the prediction error rate (Err), or the probability of assigning a future person to an incorrect ethnicity. In general, SNP selection procedures have needed to avoid estimating Err because of the large number of SNPs and the computational time required by traditional estimation methods such as cross-validation and bootstrapping. Because of some unique characteristics of our problem, we show that there is actually a simple calculation which can produce accurate estimates of the prediction error rate, allowing us to search through 1000's of SNPs relatively quickly. In addition to minimizing Err, our proposed method can also incorporate a penalty matrix so SNPs will be chosen to minimize a weighted error rate, emphasizing errors where the true and predicted ethnicities are highly dissimilar. The method also offers a means to weight populations by their prevalence in a region and estimate the true Err after selecting the SNPs. Overall, we demonstrate that a relatively small number of SNPs can accurately predict ethnicity.

Excess of X-chromosome copy number changes by microarray in women with skewed X-inactivation. *D. Warburton[1], V. Jobanputra[1], J. Kline[1,2], B. Levin[1], A. Kinney[1], O. Nahum[1], C.-Y. Yu[1], B. Levy[1]* 1) Columbia University, New York, NY; 2) N.Y. State Psychiatric Insititute, New York,NY.

Women with highly skewed X-chromosome inactivation (HSXI), defined as 85% skewing in favor of one allele, were detected during a study of the relationship between skewing and trisomic conceptions. Skewing in blood was measured by a standard HUMARA assay: for all women with HSXI we also measured the skewing in samples of left and right buccal mucosa. All women with HSXI had normal G-banded karyotypes. Because X-chromosome rearrangements are known to lead to HSXI, we looked for submicroscopic copy number changes on the X chromosome (using the Affymetrix 6.0 microarray) on 23 cases with HSXI detected in a blood sample. In 10 of the 23 women we identified at least one copy number change on the X chromosome (one deletion, 15 duplications) that showed no overlap with known variable regions. In comparison, using the same 6.0 array, only 3 of 50 women from the HapMap sample showed non-variant X chromosome changes (2 women with a duplication and one woman with 6 deletions). The proportion of women with copy number changes was significantly higher ($p<0.001$) among women with HSXI than among controls. Among the 23 women with HSXI, only 3 showed HSXI in blood and both buccal samples. Microarray analysis revealed that one of these women had a 3.3 Mb deletion in Xp22.2 containing 29 genes, including 9 identified with X-linked recessive disease (e.g., FACNB, NHS, PHKA2 and PDHA1). There was also a gene-rich duplication just proximal to the deletion. We did not identify X microaberrations in the other two women. A unique duplication in Xq13.3 was seen in 8 women with HSXI: this region contains no genes but is enriched in L1 sequences and is 4 Mb upstream of XIST. Three women had a duplication in Xp11.3 that contains a Ubiquitin gene cluster. Another woman had a duplication in Xp22.3 that includes most of the SHOX gene. None of these microduplications was identified in the 50 controls. We are currently confirming these preliminary results by analyzing additional cases with HSXI and concurrent controls from the same sample, as well as verifying the microarray findings by FISH.

SNP PGD Microarray Analysis from a Single Embryonic Cell. *W. G. Kearns[1], R. Pen[1], A. Benner[1], A. Kittai[1], E. Widra[2], R. Leach[1]* 1) Shady Grove Center for Preimplantation Genetics, Rockville, MD; 2) Shady Grove Fertility , Rockville, MD.

Objective: To amplify DNA from a single cell and to perform SNP PGD microarray analyses. We will determine total aneuploidy, identify structural chromosome aberrations and identify disease risks and copy number variations (CNVs) by a genome-wide scan. We will also determine what embryo implanted and what partner provided the extra chromosome using genotype data. **Methods:** DNA amplification was performed using a modified whole genome amplification (WGA) protocol on 398 single cells (112 WBCs, 252 human blastomeres from 37 embryos and 34 known cell lines). We used invariant DNA genomic loci to ensure the entire genome was amplified and TaqMan PCR to ensure heterozygous allele amplification. The Illumina HumanHap370 microarray was employed to determine chromosome aberrations and genotype data for ~370K SNPs. Data was analyzed with deCODE genetics Disease Miner Professional and Illumina BeadStudio software. **Results:** Our single cell analyses showed a consistent genomic coverage >90%. Our heterozygous allele detection rate >90% with a microarray detection rate and genotype call rate > 90%. We determined a complete molecular karyotype from all 252 blastomeres from 37 embryos (>5800 individual chromosomes) and all 34 cell lines (>780 individual chromosomes). Unbalanced structural chromosome aberrations were identified from all 9 cytogenetically abnormal cell lines. Genotype information was also obtained for ~370K SNPs for each cell analyzed. These genome wide scans identified disease risks from embryos for type-2 diabetes, prostate cancer, glaucoma, certain cardiovascular conditions and estrogen responsive breast cancer as well as single gene mutations. A high-resolution copy-number profile also identified CNVs throughout the genome. Using parental, embryonic and fetal genotypic data, we are also determining which partner provided the extra chromosome in aneuploid embryos and which embryo implanted. **Conclusions:** We successfully obtained complex genetic information from single embryonic cells for PGD microarray analyses. These PGD analyses can be completed for a fresh embryo transfer.

Genome-wide association study identifies FLNB and SBF2 as two novel genes underlying stature variation. *S. F. Lei[1,2], L. J. Tan[2], X. G. Liu[1,3], L. Wang[1,3], H. Yan[1,3], J. F. Liu[1], Y. Z. Liu[1], D. H. Xiong[4], J. Li[1], T. L. Yang[3], X. D. Chen[2], Y. Guo[3], F. Y. Deng[1,2], Y. P. Zhang[1,3], T. B. Jin[1,3], C. J. Papasian[1], B. M. Drees[1], J. J. Hamilton[1], R. R. Recker[4], H. W. Deng[1,2,3,4]* 1) School of Medicine, University of Missouri - Kansas City, USA 2. 3. 4; 2) College of Life Sciences, Hunan Normal University, China; 3) School of Life Science and Technology, Xi'an Jiaotong University, China; 4) Osteoporosis Research Center, Creighton University, USA.

Human stature is a highly heritable complex trait. To identify specific genes underlying human stature, a genome-wide association study was performed in 1000 unrelated homogeneous Caucasian subjects using Affymetrix arrays interrogating ~500,000 SNPs genomewide. A group of seven contiguous markers in the region of the SBF2 gene (Set-binding factor 2) are consistently associated with stature, significantly so at the genome-wide level after FDR (false discovery rate) correction (FDR q=0.034-0.042). Three SNPs in another SNP group in the Filamin B (FLNB) gene were also consistently associated with stature, significantly so with FDR q=0.042-0.048. Strong linkage disequilibrium exists among these SNPs, and two haplotype blocks were constructed in each SNP group. The haplotypes in these blocks are significantly associated with stature (e.g., point-wise testing p values are as low as 5.2×10-7). In follow-up independent replication studies, rs9834312 in the FLNB gene was consistently significantly (p=0.008), and rs10734652 in the SBF2 gene was marginally significantly, (p=0.07) associated with stature in 1,306 unrelated Caucasian subjects. In Chinese subjects, significant replication association signals were detected between rs9834312 and stature in 619 unrelated northern Chinese subjects (p=0.017), as well as between rs10734652 and stature in 2,953 unrelated southern Chinese subjects (p=0.048). These results, together with the known functional relevance of the SBF2 and FLNB genes to skeletal linear growth and bone formation, support the conclusion that FLNB and SBF2 are two novel genes underlying stature variation.

Comparison of Molecular Genetic and Anthropometric Study in a Cohort of Czech Osteogenesis Imperfecta Patients. *I. Mazura[1], I. Mařík[2], D. Zemková[3], F. Nutsu-Mazurová[4], O. Hudáková[2], P. Novosad[4], J. Zvárová[1]* 1) Dept.of Biomedical Informatics, Institute of Computer Science, Prague, Pod vodárenskou věží 2, 182 07, Prague 8, Czech Republic; 2) Ambulant Centre for Defects of Locomotor Apparatus, Olšanská 7, Prague 3, PC-130 00, Czech Republic; 3) Pediatr. Dept, University Hospital Motol, Charles University Prague, V úvalu 85, Prague 5, PC-152 00, Czech Republic; 4) Mediekos Labor Ltd, Antonínova 4464,PC-760 01, Zlín, Czech Republic.

Objectives. Aims of the study were analyse several important exons of COL1A1 gene in the group of Czech patients with diagnosis of osteogenesis imperfecta and compare the severity and progress of skeletal changes of OI types with proved molecular genetic defects. Osteogenesis imperfecta (OI) is an autosomal dominant or recessive connective tissue disease characterized by extremly high bone fragility (brittle bone disease). The incidence of this disease is approximately 1:10-50 000 newborns. Heterogenous syndrome of OI with variable phenotypic expression is defined by clinical findings . Much attention should be paid to the occurrence of mutations in the structural parts of the gene COL1A1 and COL1A2 as well as to the occurrence of single nucleotide polymorphisms in areas that are not translated into the structure of the resulting collagen protein. 52 patients with diagnosis OI type I -IV were included to this study. Patients were classified into 7 types of the OI Anthropometric parameters were useful in differential diagnostics among OI types (especially between type I and IV). We have found several molecular genetics changes e.g. nucleotide substitutions in the exon 31-36 and 39-40 of COL1A1 gene. At present we are able almost precisely characterized types of OI according to radio-clinical and anthropometric examinations but we are unable to correlate genotype and phenotype of OI patients. Acknowledgements. These results were supported by grant no. 1M06014 of Ministry of Education, Youth and Sport, Czech Republic. The research was supported by grant no.:00064203/6407 of Ministry of Health, too.

Modifying dendritic cells induces tolerance to coagulation factor VIII. *M. Seiler[1,2], V. Cerullo[1], G. Sule[1], V. Mane[1], C. Clarke[1], J. Sims[1], J. Rodgers[2, 3], B. Lee[1,2,4]* 1) Molecular and Human Genetics , Baylor College of Medicine, Houston, TX; 2) Center for Cell and Gene Therapy, Baylor College of Medicine, Houston, TX; 3) Immunology Baylor College of Medicine, Baylor College of Medicine, Houston, TX; 4) The Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX.

Hemophilia A is a blood coagulation disorder due to deficiency in Factor VIII (FVIII). About 1 in 5000 males are affected by this. Current therapy includes frequent infusions of FVIII protein. However about one third of the patients develop inhibitory antibodies to recombinant protein as a consequence of the repeated protein infusions. Genetic modification of dendritic cells (DC) is already a powerful strategy to induce immune responses to tumor antigens in autologous cell therapies. DCs induce tolerance as well as immunity, but clinical application of tolerance-inducing DC has not been developed for protein or gene therapy. Our in vitro studies show, transducing DC by helper-dependent adenovirus to express the immune suppressive cytokines TGF- and IL-10 renders them tolerogenic by, inducing T cell apoptosis, and increasing the frequency of antigen-specific regulatory T cells. Adoptive transfer of FVIII-loaded, tolerogenic DC to FVIII knock-out mice prior to gene transfer and protein treatment induced suppression of the anti-FVIII immune response, and prolonged FVIII activity. This report demonstrates that autologous cell therapy for antigen-targeted immune suppression may be developed to facilitate long-term therapy. These findings may extend to other protein and gene therapies, and potentially autoimmune diseases and solid organ transplantation.

18q12.1 Interstitial Microduplication in Mother and Child: A Case Report. *R. Veith[1], A. Swanson[2], A. Denny[2], S. Leuthner[2], A. Mitchell[3], D. Bick[2]* 1) Children's Hospital of Wisconsin; 2) Medical College of Wisconsin; 3) GeneDx.

We report a male neonate with cleft lip and palate, frontal bossing, hypertelorism, omphalocele, micrognathia, double aortic arch, diaphragmatic hernia, broad thumbs/toes. The mothers features include uterine malformation, micrognathia, hypertelorism and omphalocele. The patient and the mother share a very similar facial appearance. No other individuals in this pedigree demonstrate the phenotype. Utilizing oligonucleotide array CGH analysis we identified a genomic microduplication in the neonate and his mother comprising three genes that encode calcium-dependent glycoproteins, DSC1, DSC2 and DSC3 that are members of the desmocollin subfamily of the cadherin superfamily. This microduplication has not been observed in approximately 2200 samples using the same oligonucleotide array CGH. The duplicated segment is approximately 2 Mb and involves a contiguous region of single copy gain at 23 probe locations between the flanking positions 25185867 and 27152802 in 18q12.1. Quantitative PCR analysis using a probe in intron 11 of the DSC2 gene within the duplicated region demonstrated three gene copies in the affected child and his mother, while the paternal DNA specimen showed a normal result. Thus, the duplicated interval is maternally derived. There are two previous reports of heterozygous mutations in the DSC2 gene: one associated with familial arrhythmogenic right ventricular dysplasia/cardiomyopapthy; [1,2] the other of an apparent de novo duplication of 18q12 in 10 year old male with severe mental retardation and minor dysmorphology, seizure disorder and syndactyly. [3] Reports of larger duplications of 18q12-22 also have included cognitive deficits, seizures and dysmorphic features. [4,5] Given the similarity between the proband and his mother we believe that the 2 Mb duplication of 18q12.1 is likely responsible for their phenotype and thus represents a new microduplication syndrome. References: 1. Syrris et al., Am J Hum Genet 79:978-984, 2006 2. Heuser et al., Am J Hum Genet 79:1081-1088, 2006 3. Fryns JP et al., Hum Genet 46:341-344, 1979 4. Mewar R et al., Am J Hum Genet 53:1269-1278, 1993 5. Wolff DJ et al., Am J Hum Genet 43:A 127, 1988.

Identification of a novel cataract gene, *TDRD7*, and its role in P body-mediated post-transcriptional regulation in the lens. *S. A. Lachke[1], F. S. Alkuraya[1], I. Saadi[1], R. Cavallesco[1], A. C. Tsai[2], A. V. Drack[2], R. L. Maas[1]* 1) Division of Genetics, Brigham & Women's Hospital and Harvard Medical School, Boston, MA; 2) Human Medical Genetics Program, University of Colorado-Denver, Aurora, CO.

We have identified a novel cataract gene, *TDRD7*, in a juvenile cataract patient who carries the balanced paracentric inversion 46, XY, inv(9)(q22.33q34.11). To rapidly identify the gene involved, we used a microarray gene expression database for mouse lens development and predicted *TDRD7* as the most probable candidate among 50 genes within a 5 Mb interval around the q22.33 breakpoint. Southern blot confirmed the direct disruption of *TDRD7* in intron 2, while qRT-PCR demonstrated *TDRD7* haploinsufficiency. Furthermore, *TDRD7* knockdown in chick lenses resulted in cataract formation recapitulating the patient phenotype. Interestingly, the Tdrd7 protein localizes to cytoplasmic foci that resemble Processing or P bodies in lens fiber cells. P bodies represent a newly discovered organelle that regulates mRNA turnover in eukaryotic cells by sequestering mRNA and processing it for either storage or decay. P bodies contain RNA binding proteins and components of the microRNA machinery. Tdrd7 co-immunoprecipitates with a P body marker GW182 and its overexpression results in large P bodies confirming a role in lens P body formation. We hypothesized that *TDRD7* haploinsufficiency alters the RNA-binding specificity of lens P bodies, resulting in misregulation of lens transcripts. Consistent with this model, *Tdrd7*-knockdown in lens cells specifically leads to aberrant transcript levels of *Foxe3* and *Prox1*, two genes with key roles in lens development. We propose a model wherein *TDRD7* controls fiber cell differentiation by post-transcriptional regulation of these lens-expressed genes.

In conclusion, we describe a novel cataract gene, *TDRD7*, and a new molecular regulatory mechanism for the etiology of cataract. Our work represents the first evidence that perturbation of a tissue specific P body component results in a specific human disease. Importantly, it also suggests the possibility that tissue-specific P body components may provide a mechanism to regulate transcriptional specificity during organogenesis.

Renin-Angiotensin System Genes and Renal Function in the Multi-Ethnic Study of Atherosclerosis. *C. Y. Campbell[1], X. Guo[2], B. Fang[2], J. H. Young[1], C. A. Peralta[3], J. Coresh[1], H. J. Kramer[4], M. G. Shlipak[3], B. M. Psaty[5], S. S. Rich[6], J. I. Rotter[2], W. S. Post[1]* 1) Johns Hopkins University, Baltimore, MD; 2) Cedars-Sinai Medical Center, Los Angeles, CA; 3) University of California at San Francisco, San Francisco, CA; 4) Loyola University, Maywood, IL; 5) University of Washington, Seattle, WA; 6) University of Virginia, Charlottesville, VA.

Background: Single nucleotide polymorphisms (SNPs) in angiotensin-converting enzyme (ACE), angiotensinogen (AGT), angiotensin II type 1 receptor (AGTR1), and angiotensin II type 2 receptor (AGTR2) genes may contribute to renal function variation in the general population. Methods: 2847 participants without known cardiovascular disease in the Multi-Ethnic Study of Atherosclerosis (MESA) from 4 racial/ethnic groups (African-American, Chinese, White, and Hispanic) were genotyped. Single SNP and haplotype analyses were performed to determine the associations between genotypes and renal function, including urine albumin excretion (UAE), estimated glomerular filtration rate (eGFR) using serum creatinine and eGFR using Cystatin C. Multivariate regression analyses were used adjusting for age and gender, stratified by racial/ethnic group. Results/Discussion: 14/24 ACE, 6/10 AGT, 9/15 AGTR1, and 5/6 AGTR2 SNPs were associated with renal phenotypes ($p<0.05$ after permutation testing). 3 AGT, 3 AGTR1, and 1 AGTR2 SNPs were associated with the same phenotype in multiple racial/ethnic groups, suggesting internal replication. 2 ACE, 3 AGT, and 3 AGTR1 SNPs were associated with multiple phenotypes in the same racial/ethnic group. The AGT M235T polymorphism was associated with eGFR and UAE in African Americans (observed $p=0.02$ for continuous eGFR, $p=0.02$ for dichotomous eGFR, $p=0.004$ for continuous log UAE, $p=0.001$ for dichotomous UAE) and with dichotomous UAE in Chinese ($p=0.04$). This SNP has been shown previously to be associated with diabetic and hypertensive nephropathy. In general, haplotype data were consistent with associations identified by the single SNP analyses. These data suggest that renin-angiotensin system genes influence renal function in multiple racial/ethnic groups.

Mutation Screening of FBN1 Gene in Patients with Ascending Aortic Dilatation. *J. Šímová[1], I. Mazura[1,2], P. Čapek[2], J. Dudra[3], J. Lindner[4], J. Zvárová[1]* 1) Dept.of Biomedical Informatics, Institute of Computer Science, Prague, Prague 8, Pod vodárenskou věží 2, 182 07, Czech Republic; 2) Department of Anthropology and Human Genetics, Faculty of Science, Charles University in Prague, Viničná 7, Prague 2, 128 44 , Czech Republic; 3) Emergency Medical Service, City of Prague, Czech Republic; 4) Department of Cardiovascular Surgery, 1st Medical Faculty, Charles University, Prague, U nemocnice 2, Prague 2, Czech Republic.

Mutations in the FBN1 gene are known causes of Marfan Syndrome (MFS) and related disorders. Correlation between the genotype and the cardiovascular phenotype has not yet been estabilished. About 10 % of patients operated for aortic valve disease suffer simultaneously from ascending aortic dilatation (AAD). Cause and progression of the aortic valve disorder is considered to be the contributory factor for dilatation of primarily changed aortic wall of ascendant aorta. Our cohort contains 28 patients with ascending aortic dilatation (ranging from 35 to 76 years of age). In our study we suggest that variation in the genes encoding proteins constituting aortic wall and regulating the turnover of the extracellular matrix (e.g. FBN1, TGFBR1 and TGFBR2) are likely to influence properties of elastic fibres. We performed mutation screening of the gene for fibrillin-1 FBN1 gene. Screening involved exon 4 and exons 24-30 with their relevant intron/exon boundaries. Our gene scanning method was based on post-pcr analysis of high resolution melting curves and DNA sequrncing. We detected variation in intronic part situated close to exon 27, this variation was identified as insertion of guanine between nucleotide 37 682 and 37 683 of query sequence. We classified this mutation as IVS27 37682_37683insC. This is an initial study and although a causative link has not been shown, these data are very important for further research of the role of fibrillin-1 in relation to cardiovascular risk associated with aortic dilatation. These findings would have potential implication for risk stratification and therapeutic targeting not only for patients with existing disease but also for general population. This study was supported by grant 1M06014 of the Ministry of Education CR.

Toward a Molecular Definition of Heterochromatin. *J. A. Rosenfeld[1,2], Z. Xuan[1], R. DeSalle[3], M. Q. Zhang[1]* 1) Cold Spring Harbor Laboratory, Cold Spring Harbor, NY; 2) New York University, New York, NY; 3) American Museum of Natural History, New York, NY.

Heterochromatic regions of the genome have traditionally been defined by staining patterns or the hybridization of florescent probes. These techniques are, by definition, high-level and cannot achieve base-pair level resolution. We have utilized high-throughput Solexa sequencing data to make determinations at an individual nucleosome level in the human genome. This histone modification of H3K9me3 has been found to highly correlate with constitutively heterochromatic regions. Therefore, we obtained ChIP-seq data for H3K9me3 and mapped it across the entire genome. In order to account for the repetitive nature of heterochromatin, we have implemented a procedure for utilizing Solexa reads that map to multiple locations in the genome. A true-positive set of satellite regions was used as a control to calibrate the level of methylation needed to determine a region as ChIP-positive. We next determined the base composition preferences of the ChIP-positive regions and investigated the presence of any significant sequence motifs. This also included a determination of significant motifs at the boundaries of positive regions that may be barrier elements. Finally, we investigated the localization of H3K9me3 within the bodies of active genes.

Consumer experiences with and attitudes toward direct-to-consumer personal genome testing. *C. M. Diaz[1], T. Wang[2], S. G. Hilsenbeck[2], A. L. McGuire[1]* 1) Center for Medical Ethics & Health Policy, Baylor College of Medicine, Houston, TX; 2) Dan L. Duncan Cancer Center, Division of Biostatistics, Baylor College of Medicine, Houston, TX.

PURPOSE: This study assesses consumers experiences with and attitudes toward personal genome testing (PGT), with a focus on expectations related to the clinical integration of PGT results. METHODS: A survey of 1,087 online social networking users was conducted in April 2008 to assess (1) use and interest in PGT; (2) attitudes toward PGT companies and test results; and (3) expectations for the clinical integration of PGT. Descriptive statistics were calculated to summarize respondents characteristics and responses. RESULTS: 6% of respondents have used PGT, 64% would use PGT, and 30% would not use PGT. Of those who would use PGT, 74% would use it to gain knowledge about diseases they or their family might have. Of those who would not use PGT, 53% do not think the information would be useful and 21% have doubts regarding the reliability of PGT. 55% of all respondents think PGT will increase an individuals control over their own health and 58% think PGT will stimulate discussion about personal health within families. Of those who indicate an interest in PGT, 54% would consider testing their child, 67% would consider testing their spouse, and 43% would consider testing other family members. Those who would test their child are most likely to do so to find out if the child has a predisposition to disease. 78% of those who would consider PGT would ask their physician for help interpreting test results and 61% of all respondents believe that physicians have a professional obligation to help individuals interpret PGT results. CONCLUSION: Although PGT services are new, individuals in this sample express much interest in using these services, primarily for purposes related to their medical care. Respondents who would use PGT would seek help interpreting results from their physician and believe that physicians have a professional obligation to provide assistance. Physicians should therefore be prepared for patient demands for information, counsel, confirmatory diagnosis, preventive care, and treatment.

Cancer resistance in Down syndrome: trisomy increases survival in a sarcoma mouse model in an Ets2-independent manner. *A. Yang, R. H. Reeves* Physiology Dept., Johns Hopkins University School of Medicine, baltimore, MD.

Epidemiological studies indicate that individuals with Down syndrome (DS) get fewer solid tumors compared to the euploid population. This finding has been documented in the Ts65Dn mouse model of DS, where trisomy was found to repress adenomas in the ApcMin mouse model (T. Sussan et al., Nature 451:73, 2008). Dosage of the Ets2 gene plays a critical role in tumor repression in this model. To determine whether Ts65Dn represses multiple kinds of tumors as reported for DS, we used the NPcis tumor model. These mice lack one copy of a segment containing the Nf1 and Trp53 genes and develop a high frequency of lymphoma, sarcoma and astrocytoma due to LOH. Ts65Dn mice and a strain containing a null allele of Ets2 were crossed with NPcis mice and survival curves were plotted for four genotype groups of offspring (NPcis; Ets2+/-, NPcis; Ts65Dn, NPcis; and Ts65Dn, Ets2+/-, NPcis). As in studies with ApcMin, we found that trisomy significantly extends survival pf NPcis mice by 25%, as predicted by the repression of many types of cancer in humans with DS. In contrast to the previous study, in which increased expression of Ets2 was shown to be critical in tumor repression in the ApcMin adenoma model, the presence of one, two or three copies of Ets2 did not play a significant role in tumor repression in the NPcis model. This indicates that additional dosage sensitive genes in this trisomic region contribute to reduced cancer incidence in the Down syndrome population.

Identification of the ADIPOQ Gene Promoter and Intron 1 Variants Associated with Insulin Resistance: the NHLBI Family Heart Study. *P. An[1], M. F. Feitosa[1], J. S. Pankow[2], R. H. Myers[3], D. K. Arnett[4], P. N. Hopkins[5], J. E. Hixson[6], K. E. North[7], L. Wagenknecht[8], I. B. Borecki[1], M. A. Province[1]* 1) St Louis, MO; 2) Minneapolis, MN; 3) Boston, MA; 4) Birmingham, AL; 5) Salt Lake City, UT; 6) Houston, TX; 7) Chapel Hill, NC; 8) Winston-Salem, NC.

The ADIPOQ gene on chromosome 3q27.3 encodes adiponectin which is produced by adipocytes and recognized as a key determinant of insulin resistance (IR). Precisely which variants in ADIPOQ confer risk of diabetes and associated traits is currently unclear. We performed association tests using 809 unrelated Caucasian subjects in the NHLBI Family Heart Study (FHS). IR was estimated using homeostasis model assessment and corrected for the effects of age, gender and field center. Subjects with diabetes or taken diabetes medications were excluded from this analysis. A total of 14 SNPs in the ADIPOQ gene were genotyped in the FHS genome-wide association study, and 47 additional SNPs in ADIPOQ were imputed using Bayesian imputation-based association mapping (BIMBAM) software and HapMap data. Association tests were carried out using SAS GLM procedure under the additive model. Significant marginal associations after Bonferroni correction included variants in the promoter (rs12637534, p=0.01, 9 kb upstream from the previously reported rs17300539 and rs266729 region; and rs16861194, p=0.03, 35 bp upstream from the previously reported region). The variants rs16861194, rs17300539 and rs266729 belonged to one haplotype block but were not in LD with rs12637534. Significant associations for IR were also found at the intron 1 (near exon 1) region (rs16861205, p=0.0098) and the intron 1 (near exon 2) region (rs9877202, p=0.01). These intronic regions were in LD but did not belong to one haplotype block. Major alleles of all these SNPs were consistently associated with lower IR. Taken together, using a total of 61 genotyped or imputed SNPs covering the ADIPOQ gene, we identified two regions in the promoter and two regions in the intron 1 influencing insulin resistance in a large non-diabetic population.

DNA Copy Number Analysis, Using SNP-Based Mapping Arrays, Reveals Numerous Genomic Imbalances in Malignant Mesothelioma Cells. *M. Cheung[1], J. Pei[1], Y. Pei[1], J. Fang[1], S. C. Jhanwar[3], H. I. Pass[2], J. R. Testa[1]* 1) Human Genetics Program, Fox Chase Cancer Center, Philadelphia, PA; 2) Dept of Cardiothoracic Surgery, Division of Thoracic Surgery, New York University School of Medicine, New York, NY 10016; 3) Dept of Pathology, Memorial Sloan-Kettering Cancer Center, New York, NY 10021.

Malignant mesothelioma (MM) is a highly aggressive malignancy that arises from the serosal lining of the pleural, peritoneal, and pericardial cavities and is primarily caused by exposure to asbestos fibers. MMs are characterized by complex chromosomal alterations. To precisely identify recurrent genomic imbalances in this disease, we performed high resolution DNA copy number analysis on a series of 22 human MM cell lines. Chromosomal losses accounted for the majority of genomic imbalances. All cell lines showed homozygous or hemizygous deletions of 9p21.3, centered at the CDKN2A/ARF and CDKN2B loci. Other commonly underrepresented segments included 1p36.2-36.3, 1p22.1-22.3, 3p22.1-p21.31, 11q23.2-23.3, 13q12.2-13.2, 14q32.2, 15q15.1, 16p13.2, and 18q12.3, each observed in 52%-82% of cell lines. Loss of all or significant portions of chromosomes 22 and 4 were observed in 78% and 53% of MM cell lines, respectively, with peak levels of loss at 4q13.1, 4q34.1 and 22q12.1-12.2 in 82%-90% of the cell lines. Gain of 17q23.2 (55%) was also common. Genes located at these affected regions were analyzed by RT-PCR and genomic real time PCR to verify their status in MM cell lines. Two genes were studied further to confirm their role as tumor suppressors: Oncostatin M (OSM) and Promyelocytic Leukemia Zinc Finger (PLZF). Transfection of OSM expression constructs into MM cells or addition of recombinant OSM to the media led to a reduction in growth that correlated with decreased cyclin levels and subsequent G1 cell cycle arrest. Similarly, reintroduction of the PLZF gene into MM cells resulted in diminished cell growth. Collectively, these findings illustrate the utility of SNP-based mapping arrays for high-resolution analysis of genomic imbalances in MM and for the identification of several novel loci that may be associated with the pathogenesis of MM.

Clinical Assessment of Sequence Variants in the ClinSeq Large Scale Medical Sequencing Project. *C. Turner[1], P. Cherukuri[1], F. Facio[1], J. Teer[1], R. Cannon[2], R. Shamburek[2], J. Mullikin[1], E. Green[1], L. Biesecker[1], NISC Comparative Sequencing Program* 1) National Human Genome Research Institute, Bethesda, MD; 2) National Heart Lung and Blood Institute, Bethesda, MD.

Individualized genomic medicine promises to revolutionize the practice of medicine, though the interpretation of the vast amounts of data will be challenging. As part of the ClinSeq large scale medical sequencing project, we have sequenced 142 genes in 201 participants. A subset of three genes (LDLR, APOB and PCSK9) associated with Familial Hypercholesterolemia (FH) was used as a model for assessing pathogenicity of variants. There were 72 amplicons sequenced across these genes for the 201 participants that provided 18.1 Mbps of sequence. Comparison of these data to reference sequence identified 17 unique variants in LDLR, 62 in APOB and ten in PCSK9. To identify variants causing high-penetrance phenotypes we stratified variants as likely to be pathogenic, unlikely to be pathogenic, or uncertain. Likely pathogenic variants were nonsense, frameshift, splice (GT/AG), and those reported as causative. Unlikely pathogenic variants were common (MAF >2%) and synonymous. Uncertain variants were rare missense variants. One LDLR variant, p.Y188X, has been reported as causative for FH. Nine LDLR variants were synonymous, as were 20 in APOB and three in PCSK9. For the remaining nonsynonymous variants, we developed a position-specific amino acid substitution score reflecting the change and the conservation. Of the missense variants, six in LDLR, 19 in APOB and four in PCSK9 were not found in dbSNP. The literature suggests the p.P685L LDLR variant and the p.R3527Q APOB variant are causative. No causative variants were found in PCSK9. Analysis of the clinical data for the patients with mutations causing FH revealed markedly elevated LDL and severe atherosclerosis. These FH-causing variants were confirmed in a CLIA laboratory, and the results were returned to the participants and diagnosis and treatment for their families was instituted. These bioinformatic methods are being automated to analyze the variants in the other genes that will be sequenced in this project.

Large-scale assessment of genomic methylation in pre-implantation blastocysts. *S. Brown[1], G. Brown[2], K. Wright[1], L. Brown[1]* 1) Dept OB/GYN, Univ Vermont, Burlington, VT; 2) Columbus Regional Hospital, Columbus, IN.

A variety of evidence in both mice and humans suggests that super-ovulation and in-vitro embryo culture is associated with altered genomic methylation in resulting embryos. The data supporting this idea are largely derived from studies in which the methylation state of a small number of individual genomic loci are queried through the use of bisulfite conversion. Generally, specific loci with well-characterized methylation status (usually imprinted loci) are abnormally hypomethylated in manipulated embryos as compared with normal.

We hypothesized that if embryo manipulation results in abnormal methylation of specific loci, then the effects are likely to be generalized and present throughout the genome. In order to test this, our laboratory has developed a method for methylation-sensitive amplification of very small DNA quantities such as can be recovered from pre-implantation blastocysts. We have used this method in conjunction with micro-array analysis to comparatively assess methylation of a large number of loci in samples of trophoblast and lymphocyte derived DNA, and we present data that demonstrate the general validity of the method through bisulfite conversion analysis of specific loci.

In order to test whether super-ovulation and embryo culture results in widespread abnormal methylation, we have used our method to compare methylation of DNA from genetically identical E3.5 mouse embryos that were either the product of super-ovulation and in-vitro culture or were the product of normal mating and gestation. We have succeeded in amplifying DNA from 12 E3.5 embryos (7 experimental and 5 control), and, we present qualitative data (based agarose gel analysis of amplified products) showing evidence of widespread relative hypomethylation in the experimental group. In the next phase of this project, we will use microarray analysis to determine the degree of methylation difference between control and experimental embryos. We believe that our analysis will lead a better understanding of the effects of super-ovulation and embryo manipulation on genomic methylation.

Analysis of the Methyltetrahydrofolate reductase and Glutathione S-transferase omega-1 genes as modifiers of the cerebral response to ischemia. *L. Peddareddygari[1], A. V. Dutra[1], M. Levenstien[2], S. Sen[3], R. P. Grewal[1]* 1) New Jersey Neuroscience Institute at JFK Medical Center, 65 James Street, Edison, NJ 08818; 2) Program in Cancer Biology and Genetics, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10065; 3) UNC Hospital Stroke Center 7003A Neuroscience Hospital, CB# 7025, Chapel Hill, NC 27599.

Stroke is the third leading cause of death and the most common cause of disability in North America. There is increasing evidence of the role of genes as independent risk factors in the development of stroke. However, there have been few investigations of the potential role of genes in the response of the brain to ischemia. Cerebral ischemia involves a complex cascade of reactions in brain tissue and ultimately, in non-lacunar stroke, the volume of infracted tissue is influenced by many factors including vascular distribution, collateral supply and the mechanism of stroke (cardioembolic versus large artery atherosclerosis). The volume of a stroke is important because, in general, it correlates with resultant disability. We hypothesize that polymorphisms in the genes encoding those proteins involved in these reactions could act as modifiers of the response of cerebral tissue to ischemic injury and influence infarct size. We studied the effect of two genes, Methyltetrahydrofolate reductase (MTHFR) and Glutathione S-transferase omega-1 (GSTO1). The selection of these two genes is based upon biological plausibility as key participants in oxidative mechanisms in the cerebral response to ischemia. We analyzed the C677T polymorphism (in the MTHFR gene) and the C419A polymorphism (in the GSTO1 gene) in 134 patients with non lacunar ischemic stroke in whom the mean stroke volume was 71.37 cm3 (S.D 90.1 cm3). To detect the effect of the MTHFR C677T and GSTO-1 C419A polymorphisms on stroke volume, ANOVA and generalized linear model analysis was performed. We found no significant influence of the MTHFR (p=0.72) or GSTO-1 (p=0.58) polymorphisms on the stroke volume. Our study does not support a major gene affect of either of these genes as a modifier of the response of the brain to ischemia.

Detecting BRCA2 Protein Truncation in Breast Tissue Biopsies to Identify Hereditary Cancer. *J. Holt[1,3], R. Lieberman[1], C. Snyder[2], V. J. Clark[3], H. Lynch[2]* 1) University of Colorado Health Sciences Center, Aurora, CO; 2) Creighton University, Omaha, NE; 3) Tissue Genetics Inc, Aurora, CO.

Mutations in the BRCA2 gene are dominantly inherited, but cancer occurs when the wildtype allele mutates, resulting in LOH (loss of heterozygosity) within the cancer. Identifying breast cancer patients with hereditary BRCA mutations will enable appropriate treatment and screening, while non-carrier family members can follow general population screening guidelines. Because most disease-associated BRCA2 mutations are truncating mutations, a test for truncated BRCA2 proteins should identify most BRCA2 hereditary cancers. We have developed a test to identify truncated BRCA2 proteins in breast cancer tissue biopsies by direct immunohistochemistry, without amplification or genetic manipulations. N-terminal and C-terminal antibodies are used to visualize protein truncation by demonstrating that the beginning of the protein is present but the end (terminus) is absent. A quantitative C-terminal immunostaining score (a C/N terminal truncation ratio) correctly classified 20/21 breast cancers from BRCA2 mutation carriers and 57/58 sporadic breast cancers. This is a sensitivity of 95% and specificity of 98% for the test. Due to the presence of C-terminal BRCA2 protein and atypical clinical features of the misclassifed cancer in a BRCA2 mutation carrier, we performed DNA sequence analysis on this cancer. The results showed continued presence of the BRCA2 wildtype allele in the cancer, indicating that this is a sporadic cancer which occurred in a mutation carrier. Our immunohistochemistry based test (which takes only 4 hours) appears to identify BRCA2 hereditary cancer with high accuracy. The test also appears to diagnose the biochemical loss of BRCA2 protein in cancers (BRCA2 mutant genotype) which will usually but not always agree with the presence of a germline BRCA2 mutation detected by DNA sequencing of blood samples. This rapid but accurate tissue test can diagnose hereditary BRCA2 breast cancer at the time of biopsy, allowing medical oncologists to implement targeted therapies or timely prophylactic surgery without the usual four-week wait for DNA sequencing results.

Branchio-Oculo-Facial syndrome caused by a mutation in the TFAP2A gene. *R. Klatt[1], D. Chitayat[1], W. Reardon[2], J. Milunsky[3]* 1) Div Clinical Genetics, Hosp Sick Children, Toronto, ON, Canada; 2) Our Lady's Hospital for Sick Children, Crumlin, Dublin, Ireland; 3) Center for Human Genetics, Boston University School of Medicine, Boston, Massachusetts, USA.

Branchio-Oculo-Facial (BOF) syndrome (MIM 113620) is a rare autosomal dominant condition characterized by hypertrophy of the lateral pillars of the philtrum which look like a poorly repaired cleft, a broad, asymmetric nose with a broad root and tip, lacrimal duct obstruction and a branchial sinus and/or linear skin lesion behind the ear. We report on a 11-year-old male with typical features of BOF syndrome. The antenatal history was negative for exposure to maternal illness or known teratogens. The family history was negative for recognizable genetic syndromes. The patient was born at term by spontaneous vaginal delivery and a birth weight of 3.5 kg. At birth, a bilateral cleft lip and hemangiomatous areas behind both auricles (left more prominently than right) were noted. Additional dysmorphisms noted were a short forehead, heavy eyebrows, broad nasal root and tip, bilateral scars of repaired cleft lip, thin upper lip with prominent lower lip, and low-set ears. The patient also had conductive hearing loss and myopia. CT scan of the petrous bone showed deficiency of the long process of the incus on the right and a bony plate overlying the oval window on the left. Development was normal. The patient had a normal male karyotype. DNA analysis of the TFAP2A gene revealed a mutation (R254W) within exon 4. TFAP2A missense mutations have been detected in four patients with BOF. The exon 4 mutation described is within the central basic DNA binding region. TFAP2A knockout mice demonstrate impairment in neural crest-derived facial structures. The TFAP2A gene is part of the AP-2 family of transcription factors that regulate gene expression during embryogenesis of the eye, face, body wall, limbs, and neural tube. Specifically, this gene has been shown to regulate the development of the facial prominences, limb buds, cranial closure, and development of the lens vesicle, abnormalities detected in patients with BOF syndrome.

Modeling SNP Genotype Data with Informative Missingness in Samples of Unrelated Individuals. *N. Liu* Dept Biostatistics, Univ Alabama at Birmingham, Birmingham, AL.

Even with the advancement of modern technology, data with missing genotypes are still common in genetic studies. Besides the possibility of causing missing from equipment itself, such as any damage or loss of performance of some probes of the multi-plexed platforms used for genotyping, there are other situations where missing can also be induced, such as variation in DNA quality or molecular effects, experimental techniques (including genotype calling algorithms) used, and the conduct of studies can cause some individuals (e.g. cases versus controls in the case-control studies) and some sites to have more or less than their fair share of missing data. Although some statistical methods can handle missing data, they usually assume that genotypes are missing at random either explicitly or implicitly, that is, at a given marker, different genotypes and different alleles are missing with the same probability. In this study, we demonstrate that the violation of this assumption may lead to serious bias in allele frequency estimates, and association analysis based on this assumption can be biased. To address this limitation in the current methods, we propose a novel missing data model which can estimate allele frequency and missing rate without assumption about the missing data distribution. Analytically, we prove that the allele frequency and missing probability are identifiable under our model. Empirically, simulation studies illustrate that our proposed model can reduce the bias both for allele frequency estimates and association analysis due to incorrect assumption on the missing data mechanism. In addition, we evaluate the impact of departure from Hardy-Weinberg equilibrium on the model. Lastly, we illustrate the utilities of our method through its application to HapMap data and another real data.

A Case of Goltz Syndrome Presenting with a Vascular Type Amniotic Band Sequence Phenotype. *J. A. Defant[1], H. J. Stalker[1], C. A. Williams[1], N. Knutson[2], A. I. Dagli[1], R. T. Zori[1]* 1) University of Florida Division of Genetics and Metabolism Gainesville, FL; 2) University of Florida Division of Neonatology Gainesville, FL.

Goltz syndrome is a rare X-linked dominant genetic condition characterized by distinctive skin abnormalities and other birth defects affecting multiple systems including: craniofacial, skeletal, urinary, gastrointestinal, cardiovascular and central nervous systems. The clinical manifestations are extremely variable and the recent identification of the PORCN gene has helped define the clinical expression of this condition. We present a female African-American infant born with multiple congenital anomalies including: external ear dysplasia, bilateral cleft lip and palate, Tessier clefts (types 4 and 7), cleft sternum, bilateral microphthalmia, omphalocele, bladder exstrophy and genital anomalies. Limb anomalies included: ectrodactyly of the right hand and foot, small appendage in place of left thumb and right 3-5 toe syndactyly. No structural anomalies of the brain or heart were noted. Some of the anomalies resembled disruptions that might otherwise have been considered to be amniotic band effects. At birth, the infants skin had generalized scaling and areas of reddish hypopigmentation. Some of these areas appeared atrophic. At a few weeks of life, some of the atrophic areas were following the lines of Blaschko. Sequencing of the PORCN gene identified a heterozygous c.178G>A nucleotide change, previously reported as pathogenic, that confirmed a diagnosis of Goltz syndrome. To our knowledge, this is the most severe presentation of Goltz syndrome with a documented PORCN mutation. This case illustrates the wide range of pigmentary changes that can be seen in this condition and illustrates the severe extent to which malformations, some resembling vascular disruption events, can occur in the syndrome.

Detection of mosaic *RB1* mutations in families with retinoblastoma. *B. Piovesan[1], D. Rushlow[1], K. Zhang[1], N. L. Prigoda[1], M. N. Marchong[2], R. D. Clark[3], B. L. Gallie[1,2]* 1) Retinoblastoma Solutions, Toronto Western Hospital, Toronto, Ontario, Canada; 2) Division of Applied Molecular Oncology, Ontario Cancer Institute/Princess Margaret Hospital, University Health Network, Toronto, Ontario, Canada; 3) Division of Ophthalmology, Childrens Hospital Los Angeles, Los Angeles, CA, USA.

We fully analyzed the *RB1* gene in 1,020 retinoblastoma families and increased our mutation detection rate for bilaterally affected probands from 92.6% to 94.8% by including an allele-specific PCR (AS-PCR) assay, sensitive enough to reveal low-level mosaicism for any of eleven recurrent RB1 CGA>TGA nonsense mutations. For unilateral tumors we detected both oncogenic changes in 92.7% of sporadic unilateral tumors (357/385) and determined that 14.6%(52/357) of unilateral probands, with both tumor mutations identified, carried a tumor mutation in blood. Mosaicism was evident in 5.5% of bilateral probands, in 29% of unilateral probands with constitutional mutations and in one unaffected mother of a unilateral proband. Half of the mosaic mutations were identified only with the help of the AS-PCR test for the eleven recurrent mutations, which detects mosaic mutations at levels between 1% and 15%, not usually detectable by standard sequencing methodology. This suggests that a significant number of low-level mosaics with non-recurrent RB1 mutations remain unidentified by current technology. We show that the use of linkage analysis in a two-generation retinoblastoma family resulted in the erroneous conclusion that a child carried the parental mutation, because the founder parent was mosaic for the RB1 mutation. We also show that almost 10% of unaffected parents of sporadic constitutional probands carried heterozygous reduced penetrance mutations, but only 0.7%(one unaffected parent) showed mutational somatic mosaicism.

Congenital high airway obstruction and gastrointestinal atresia: A new syndrome? *R. J. Silver[1], S. Keating[2], S. Pantazi[3], P. Shannon[2], H. Sroka[1], D. Chitayat[1,4]* 1) Prenatal Diagnosis & Medical Genetics, Mount Sinai Hospital, Toronto, Canada; 2) Dept of Laboratory Medicine & Pathobiology, Mt Sinai Hospital, Toronto, Canada; 3) Dept of Diagnostic Imaging, Mt Sinai Hospital, Toronto, Canada; 4) Division of Clinical Genetics & Metabolics, Hospital for Sick Children, Toronto, Canada.

Congenital High Airway Obstruction Syndrome (CHAOS) is a rare condition that is usually lethal in fetuses. Characteristic fetal ultrasound findings secondary to the obstruction include enlarged echogenic lungs, dilated airways, flattened/inverted diaphragm, polyhydramnios, and ascites. Most cases are sporadic with unknown etiology, though cases with chromosome abnormalities as well as single gene disorders have been reported. Here we report on a case of CHAOS associated with multiple gastrointestinal atresias. The patient is a 27-yr-old G1 woman of Indian descent; her husband is of the same descent. Their family history is non-contributory and there is no history of consanguinity. Fetal ultrasound at 20.7 and 21.7 wks gestation showed ascites and large, hyperechoic lungs with inverted diaphragm (consistent with CHAOS), a dilated stomach and proximal duodenum (suggestive of duodenal atresia), and a two-vessel cord. Fetal MRI at 22.3 wks gestation showed findings consistent with CHAOS (laryngeal obstruction measuring 5mm, diaphragm inversion and lung hyperinflation, centrally compressed heart, dilated airways). Additionally, there were findings suggestive of duodenal atresia and possible anal atresia. The couple elected to terminate the pregnancy. Autopsy confirmed laryngeal atresia with expanded lungs, duodenal and anal atresias, and possible esophageal atresia. Other findings included a small spleen, heterotopic pancreas, midline liver, ascites, and two-vessel cord. The remainder of the anatomy, including the brain, was normal. Chromosome analysis revealed a normal male karyotype, and microarray analysis did not reveal microdeletions or duplications. The etiology remains unknown. This case represents a novel association of laryngeal atresia with multiple gastrointestinal atresias and may be a hitherto new syndrome.

HBA Mutations Detected by MLPA in -thalassemia Patients. *H. Onay[1], A. Ekmekci[1], E. Ataman[1], M. Akgul[1], A. Vahabi[1], Y. Aydinok[2], C. Vergin[3], F. Ozkinay[1,2]* 1) Dept Medical Genetics, Ege Univ, Izmir, Turkey; 2) Dept Pediatrics, Ege Univ, Izmir, Turkey; 3) Dr Behcet Uz Children Hospital, Izmir, Turkey.

Thalassemia syndromes are the most common monogenic disorders in humans and -thalassemia major is one of the most important public health problem in Turkey. Many genetic factors play roles in modifiying the disease severity such as mutations in the alpha globin gene (HBA), UGT1 gene (bilirubin metabolism) or HFE gene (iron metabolism). Deletions or duplications in HBA gene determine the cumulation of chain in -thalassemia major patients. The human alpha globin gene cluster is located on chromosome 16 and deletions or duplications are the most important mutations. Multiple ligation dependent probe amplification (MLPA) technique is a simple and rapid technique and used in many genetic disorders in which deletions and duplications are common. In this study we used a commercial MLPA kit for detecting alpha globin gene mutations. The kit contains 24 different probes in the HBA region. In this study we aimed to investigate the incidence of alpha globin gene mutations using MLPA technique in 19 -thalassemia major patients which were diagnosed in our department. Alpha globin gene deletions were detected in 2 out of 19 (10,5%) -thalassemia major patients. In one of the patients the deletion was located between the HBA2 gene and HBA1P gene. The deletion detected in the other patient was located between the HBA1 gene and HBA2 gene. This result suggests that alpha globin gene mutations in -thalassemia major patients are not rare. MLPA technique is suitable to investigate the mutations in this complex gene cluster.

Pre And Postnatal Features Of Edwards Syndrome Due To A Novel Partial Deletion/Duplication of Chromosome 18. *S. Kirmani, D. Babovic-Vuksanovic, C. Hutchens, G. Velagaleti* Mayo Clinic College Of Medicine, Rochester, MN.

Most cases of Edwards syndrome are due to full trisomy 18, with only 2% of cases having partial trisomy 18. We describe pre and postnatal findings in a case with a novel partial deletion/duplication of chromosome 18, with severe phenotypic features of Edwards Syndrome. A 26-year-old G2P1001 female underwent amniocentesis, since ultrasound at 24 weeks gestation showed a fetus that was small for gestational age, with a VSD and a single umbilical artery. Rocker-bottom feet and a hypoplastic cerebellum were seen on subsequent scans. Chromosome analysis revealed an abnormal karyotype: 46,XX,der(18)dup(18)(q21.3q11.2)del(q21.3). Subtelomere and locus specific FISH revealed dup(18)(18pter+,cen18+,MALT1++,BCL2-,18qter-). Both parents had a normal karyotype. The baby was born via induced vaginal delivery at 38 4/7 weeks gestation. Birth weight was 1.92 kg and length was 43 cm (both << 3 %ile). Apgars were 3, 5 and 6 at 1 5 and 10 minutes. Mechanical ventilation was initiated due to poor respiratory effort. On exam, she had hypertelorism, epicanthal folds and upslanting palpebral fissures. The nose was short with an upturned tip, the ears were small, low-set and overfolded, and a cleft palate was noted. Cardiac exam revealed a harsh grade 3/6 systolic murmur. She had a single umbilical artery, and genital exam revealed hypoplastic labia majora. Both hands were short with proximally implanted thumbs, cutaneous partial 2-3 syndactyly, tapering digits, and 5th finger clinodactyly. She had bilateral club feet and rocker-bottom deformity. Echocardiogram showed 2 large VSDs, and tricuspid valve dysplasia.. Respiratory support was withdrawn, and the baby died at 3 days of age. De novo partial deletion/duplication of 18q resulting in phenotypic features of Edwards Syndrome has not previously been described. Molecular mapping of the Edwards Syndrome phenotype has implicated 2 non-contiguous regions, 18q21.1-qter and 18q12.3-q22.1, with duplications of the latter region associated with a severe phenotype. Our results confirm these findings, and suggest that prognostic information can be given based on the region duplicated on 18q.

MUC5AC is a Candidate Gene for Familial Interstitial Pneumonia. *A. Wise[1], M. Speer[2], M. Steele[2], L. Burch[3], A. Herron[2], J. Loyd[4], K. Brown[1,5], J. Phillips III[4], S. Slifer[6], T. Sporn[2], P. McAdams[2], M. Schwarz[1,5], D. Schwartz[1]* 1) National Jewish Medical and Research Center, Denver, CO; 2) Duke University Medical Center, Durham, NC; 3) National Institute of Environmental Health Sciences, Research Triangle Park, NC; 4) Vanderbilt University School of Medicine, Nashville, TN; 5) University of Colorado Health Sciences Center, Denver, CO; 6) University of Miami, Miami, FL.

The Idiopathic Interstitial Pneumonias (IIPs) are complex conditions, with limited treatment options and unknown etiology. Previously, a whole genome screen of 82 families with FIP (the familial form of IIP), found linkage to the p-ter end of chromosome 11 with a LOD score of 3.3. Follow-up association testing revealed one SNP in particular, RS7944723 with a p-value=1 x 10-6 in familial cases versus controls and p-value=0.002 in sporadic cases versus controls. RS7944723 lies in the mucin gene MUC2, however, markers within MUC2 and MUC5AC exhibit strong LD and no markers were typed in MUC5AC (one of the predominant mucins expressed in the airways). Therefore, both MUC2 and MUC5AC were sequenced. Sequencing of both genes resulted in 34 significant SNPs with p-values < 0.05 in either familial or sporadic cases from MUC2 and MUC5AC. However, only in MUC5AC were 6 SNPs found that were significant in both familial and sporadic cases. Thus, further genotyping of a subset of 9 promising SNPs in MUC5AC was conducted using additional familial cases and spouse controls, along with an entirely separate replicate cohort of sporadic patients. All 9 of the SNPs tested to date have replicated the previous results, strongly implicating mutations in the mucin genes in both familial and sporadic IIP. Moreover, a strong association was found for a haplotype containing 4 SNPs in both familial and sporadic cases (p-value 0.002 and 0.001 respectively). While the SNP in MUC2 is intronic, all 3 MUC5AC SNPs produce amino acid changes. Thus, non-synonymous polymorphisms in MUC5AC are associated with both familial and sporadic cases of IIP.

Genetic variants associated with blood lipids levels in the U.S. population: Third National Health and Nutrition Examination Survey. *M. Chang[1], A. Yesupriya[1], RM. Ned[1], NF. Dowling[1], PW. Mueller[2]* 1) National Office of Public Health Genomics, CDC, Atlanta, GA; 2) National Center for Environmental Health, CDC, Atlanta, GA.

Identification of genetic variants related to blood lipid levels using a large, nationally representative population-based survey may allow for a better understanding of the genetic contribution to serum lipid levels in the major race/ethnic groups of the U.S. population. We examined the association of 22 polymorphisms in 13 candidate genes [including *ABCB1* (rs1045642), *ADH1C* (rs698, rs1693482), *ADRB2* (rs1042713, rs1042714), *ADRB3* (rs4994), *APOE* (rs7412, rs429358), *ITGB3* (rs5918), *MTHFR* (rs1801131, rs1801133, rs2066470), *MTRR* (rs1801394), *NOS3* (rs1799983, rs2070744), *SERPINE* (rs1799762), *PON1* (rs662, rs854560), *PPARG* (rs1801282), *TNF* (rs1800750, rs1800629, rs361525)] with serum high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LCL-C), total cholesterol (TC), and triglycerides (TG) in individuals aged 17 years and older (n=6,016) from the second phase (1991-1994) of the Third National Health and Nutrition Examination Survey (NHANES III). Univariate and multivariable linear regression analyses were used to test for genetic associations assuming an additive mode of inheritance for the three major race/ethnic groups in the U.S. (non-Hispanic white, non-Hispanic black, and Mexican-American). *APOE* (rs7412 and rs429358) was significantly associated ($p<0.05$) with LDL-C and STC levels in all three ethnic groups after adjustment for demographic, environmental, and behavioral factors (age, sex, education, alcohol intake, smoking status, physical activity, body mass index, dietary fat intake). In addition, variants in *ABCB1*, *ITGB3*, *MTHFR*, *NOS3*, *PON1*, and *TNF* were found to be associated with one or more blood lipid measures in at least one race/ethnicity group. This is the first study to describe genetic associations with blood lipid levels in a nationally-representative sample of the U.S. population. These results may provide some insight into the biological mechanisms underlying serum lipid and cholesterol concentrations.

Constitutional 11p15 imprinting center mutations, epimutations and uniparental disomy cause non-syndromic Wilms tumor. *R. H. Scott[1], J. Douglas[1], L. Baskcomb[1], N. Huxter[1], K. Barker[1], A. Craft[2], M. Gerrard[2], J. Kohler[2], G. Levitt[2], S. Picton[2], B. Pizer[2], M. Ronghe[2], D. Williams[2], J. Cook[3], P. Pujol[4], E. Maher[5], J. Birch[6], C. Stiller[7], K. Pritchard-Jones[2], N. Rahman[1], The Factors Associated with Childhood Tumours (FACT) Collaboration* 1) Section of Cancer Genetics, Institute of Cancer Research, Sutton, UK; 2) Childhood Cancer and Leukaemia Group (CCLG), UK; 3) Department of Clinical Genetics, Sheffield Children's Hospital, Sheffield, UK; 4) Service de Génétique Médicale, Centre Hospitalier Universitaire, Montpellier, France; 5) Department of Medical and Molecular Genetics, University of Birmingham, Birmingham, UK; 6) CRUK Paediatric and Familial Cancer Research Group, Royal Manchester Children's Hospital, Manchester, UK; 7) Childhood Cancer Research Group, University of Oxford, Oxford, UK.

Constitutional abnormalities at the imprinted 11p15 growth regulatory region cause syndromes characterised by disordered growth, some of which include a risk of Wilms tumor.We explored their possible contribution to non-syndromic Wilms tumor using methylation-specific MLPA. This identified constitutional 11p15 abnormalities in in genomic lymphocyte DNA from 13 of 437 individuals (3%) with sporadic Wilms tumor without features of growth disorders, including 12% of bilateral cases (P=0.001) and in one familial Wilms tumor pedigree. No abnormality was detected in 220 controls (P=0.006). Abnormalities identified included H19 DMR epimutations, uniparental disomy 11p15 and two novel H19 DMR imprinting center mutations (one microinsertion and one microdeletion).Our data identify microinsertion as a new class of imprinting center mutation and provide insights into mechanisms of imprinting disruption. In addition, they identify constitutional 11p15 defects as one of the commonest known causes of Wilms tumor and reveal clinically important epigenotype-phenotype associations. The impact on clinical management dictates that constitutional 11p15 analysis should be considered in all individuals with Wilms tumor.

COMPLEX CHROMOSOMAL REARRANGEMENT IS REVEALED BY CGH-A IN A GIRL WITH MULTIPLE CONGENITAL ANOMALIES. *M. Michelson[1,2], C. Vinkler[1,2], I. Linder[2,3], M. Yanoov-Sharav[1,2], T. Lerman-Sagie[2,3], D. Lev[1,2]* 1) Genetics Inst, Wolfson Medical Ctr, Holon, Israel; 2) Metabolic Neurogenetic Clinic,Wolfson Medical Ctr, Holon, Israel; 3) Pediatric Neurology Unit,Wolfson Medical Ctr, Holon, Israel.

Structural chromosomal aberrations are often associated with multiple congenital anomalies (MCA) and various syndromes. Cryptic microduplications and microdeletions undetected by routine karyotype analysis can be revealed by CGH-microarray and help both in diagnosis and proper genetic counseling. A 9 month old baby girl presented to our clinic with developmental delay,congenital heart defect(VSD), nephrolithiasis, cholelthiasis and dysmorphic features.She has hypertelorism,upslanting palpepbral fissures,arched eyebrows, epicantal folds and abnormal structure of her right external ear. During pregnancy single umbilical artery and hyperechogenic bowel were found by ultrasound examination. Amniocentesis was done and a normal 46,XX karyotype was found. She is the second child of an unrelated generally healthy couple. They have a healthy six year old girl and a history of a previous pregnancy which was terminated because of severe cleft lip and palate. Due to MCA CGH-microarray was performed on DNA from lymphocytes, using BAC clones. This analysis detected single copy gain of 12 BAC clones from the proximal region of the long arm of chromosome 13q12.11q12.12. A second abnormality was also detected and characterized by a single copy gain 4 BAC clones from the pericentromeric region of the short arm of chromosome 20 at 20p11.21. Fluorescence in situ hybridization (FISH) analysis using BAC clones from the 13q12.11 region and 20p11.21 identified two marker chromosomes. These marker chromosomes were seen in 28 of 30 cells examined (93%) and result in partial mosaic trisomy for proximal 13q and pericentromeric 20p. Neither parent carried a translocation or marker chromosome for either 13q or 20p. This case emphasizes the importance of further investigation in cases of multiple congenital anomalies in spite of normal karyotype in amniocytes or lymphocytes. Molecular cytogenetic analysis by CGH-microarray allowed both diagnosis and accurate counseling to this family.

Follow up of the genome-wide association study in celiac disease - identification of two novel shared immune loci.

A. Zhernakova[1], G. Trynka[2], J. Romanos[2], K. A. Hunt[3], A. Ryan[4], G. Turner[4], L. Franke[1,2], C. C. van Diemen[2], M. Bruinenberg[2], D. Barisani[5], R. McManus[4], D. A. van Heel[3], C. Wijmenga[1,2] 1) Dept Biomedical Gen, UMC Utrecht, The Netherlands; 2) Genetics Department, UMC Groningen, The Netherlands; 3) Barts and the London, School of Medicine and Dentistry, London, United Kingdom; 4) Department of Clinical Medicine, Institute of Molecular Medicine, Trinity College Dublin, Ireland; 5) Department of Experimental Medicine, University of Milano Bicocca, Italy.

Celiac disease is a common food intolerance, affecting 1% of Caucasians and caused by an abnormal immune reaction against gluten - a dietary protein present in wheat, barely and rye. Recently we performed a genome-wide association study (GWAS) in 767 celiac cases and 1422 controls, that was followed up by replication of the top-1020 associated SNPs in an extended cohort of >5000 celiac cases and controls from three populations. We identified 8 novel celiac genes, most of which are involved in immune response and a number of these are shared risk factors with other autoimmune and inflammatory disorders. Interestingly, from the 8 genes, only 3 were identified by SNPs ranking in the top 100 of p-values in the original GWAS, whereas 3 loci were presented by SNPs ranking below the top-800 in the original study. Therefore we decided to extend the GWAS replication further by genotyping an additional 650 SNPs in the replication cohort of 5000 celiac cases and controls. In a combined analysis of 7200 subjects (GWAS + replication cohorts), 13 novel non-HLA SNPs showed association with p(CMH)<10E-05. The top 12 SNPs were further typed in 545 Italian celiac cases and 593 controls. Combined analysis in >8300 subjects indicate association of 2 loci with pCMH of 1.3E-08 and 5.2E-07 respectively. Both associated loci are not unique for celiac, but confer also susceptibility to rheumatoid arthritis and type 1 diabetes (locus 1) and inflammatory bowel disease (locus 2). By extension of the GWAS-follow up in a large collection of samples, we have discovered two novel shared autoimmune loci. The extensive follow up of GWAS is a promising strategy for identification of novel genes in complex disorders.

***SMN1* allele frequencies in the major ethnic groups within North America.** *B. C. Hendrickson[1], C. Donohoe[1], V. Akmaev[1], E. Sugarman[1], P. Labrousse[1], L. Boguslavskiy[1], K. Flynn[1], E. M. Rohlfs[1], B. Allitto[1], C. Sears[2], T. Scholl[1]* 1) Genzyme Genetics, Westborough, MA; 2) Department of Hematology and Oncology, Childrens Hospital, Boston, MA.

Spinal Muscular Atrophy (SMA) is the most common inherited lethal disease of children. Various genetic deletions involving the loss of *SMN1* exon 7 are reported to account for 94% of mutant alleles that convey this recessive trait. Published literature places the carrier frequency for *SMN1* mutations between 1 in 25 and 1 in 50 in the general population. Although SMA is considered to be a pan-ethnic disease, carrier frequencies for specific ethnicities are unknown. To provide an accurate assessment of *SMN1* mutation carrier frequencies in African American, Ashkenazi Jewish, Asian, Caucasian, and Hispanic populations, more than 1000 anonymous specimens in each ethnic group were tested using a clinically validated, quantitative real-time PCR assay that measures exon 7 copy number.

Differences in the frequency of carriers with only 1 copy of *SMN1* were significant between several ethnic groups. The 1 copy genotype frequency in Caucasians was 1 in 37 (2.7%; 95%CI: 1.9%, 3.9%), 1 in 46 (2.2%; 95% CI: 1.5%, 3.3%; P=0.4762) in Ashkenazi Jews, 1 in 56 (1.8%; 95% CI: 1.1%, 2.8%; P=0.1393) in Asians, 1 in 91 (1.1%; 95% CI: 0.6%, 1.9%; P=0.0089) in African Americans, and 1 in 125 (0.8%; 95% CI: 0.4%, 1.5%; P=0.0007) in Hispanics (P value = significance of 1 copy genotype frequency difference from Caucasian). Also, differences in the frequency of genotypes with three or more *SMN1* copies were observed.

These data refine the knowledge of SMA by specifically assessing mutation carrier frequencies within the major ethnic groups within North America at a high level of accuracy afforded by large data sets. These results support recent recommendations by some researchers and family support groups for more widespread SMA carrier screening by expanding the information base available to clinicians and patients considering testing.

Brachydactyly Type D and Type E linked to chromosome 7p in an ethnic group from eastern Nepal. *K. D. Williams[1], J. Blangero[2], S. A. Czerwinski[1], C. R. Cottom[1], S. Lawrence[1], J. Subedi[3], B. Jha[4], T. Dyer[2], J. L. VandeBerg[2], S. Williams-Blangero[2], B. Towne[1]* 1) Wright State University Boonshoft School of Medicine, Dayton, OH; 2) Southwest Foundation for Biomedical Research, San Antonio, TX; 3) Miami University, Oxford, OH; 4) Tribhuvan University Institute of Medicine, Kathmandu, Nepal.

There is phenotypic overlap between Brachydactyly Type D (BDD) and Brachydactyly Type E (BDE) that suggests a possible common underlying etiology. BDD is characterized by a short, broad distal phalanx of the thumb, and BDE includes short 3rd, 4th, and/or 5th metacarpals and often the thumb. The Jirel ethnic group of eastern Nepal participates in various genetic epidemiological studies, including those of skeletal growth and development. As part of the Jiri Growth Study, a hand-wrist x-ray is taken annually for each child to assess skeletal maturation. Hand-wrist x-rays have also been taken for all adult relatives in order to study familial brachydactylies. For this study, a total of 2,308 individuals (1,381 children; 927 adults) in one large extended pedigree were examined for BDD and BDE. The prevalence of BDD and BDE in this sample is 3.94%. We used variance-components methods implemented in SOLAR (Almasy and Blangero, 1998) to conduct a genome-wide linkage scan for QTL influencing BDD- and/or BDE-affected status in a subsample of 1,722 individuals typed for ~400 STR markers. The additive genetic heritability was highly statistically significant in this sample (h^2 SE = 0.78 0.11, $p=2\times10^{-12}$). Significant linkage was found for BDD and/or BDE to markers on chromosome 7p at 40 cM between 7p21-7p14 (LOD score = 3.74). Possible positional candidate genes in the one-lod support interval include *TWIST1* and the *HOXA1-A13* cluster. Mutations of *TWIST1* and *HOXA13* have been implicated in other limb development disorders. This is the first study to report significant linkage results for BDD and BDE using a large extended pedigree, and the first to suggest that *TWIST1* and/or the *HOXA1-A13* cluster may contribute to these specific skeletal anomalies. Supported by NIH grants F32HD053206, R01HD40377, R01AI37091, R01AI44406, and R37MH59490.

Limitations on the predictive power of combining genetic markers. *M. Mosteller, L. Li, I. Grossman, M. R. Nelson*
Genetics, GlaxoSmithKline, Res Triangle Pk, NC.

Substantial research effort is being applied to the discovery of predictive genetic markers with the intent of estimating the probability that a patient will develop a disease or respond a particular way to a drug treatment. The increasing application of whole genome genotyping technologies has resulted in the identification of numerous individual genetic risk factors for diseases and clinical responses. These markers are statistically associated with disease status or treatment response, but may lack sufficient predictive power to be clinically useful. In these situations, we are interested in the opportunities for combining genetic markers to generate a prognostic test with useful predictive power. We have investigated the conditions under which the composite sensitivity and specificity (SE and SP, respectively) of combinations two independent markers will both exceed the sensitivities and specificities of the contributing markers (SE1, SE2 and SP1, SP2, respectively). When we classify marker genotypes as either high risk (+) or low risk (-), there are four possible two-marker combinations: ++, +-, -+, and --. If, for example, the -- combination were predictive of individuals at low risk and all other combinations were predictive of high risk, then it can be shown that while the sensitivity of the composite test may be as large as the sum of SE1 and SE2, its specificity will be no greater than the lesser of SP1 and SP2. Only under restricted conditions - conditions that depend on the presence of epistatic genetic effects and the relative frequencies of the high risk genotypes - can composite sensitivity and specificity be greater than those of the contributing markers. However, in most circumstances, the prospects for using combinations of multiple markers to substantially improve the predictive power of markers with moderate effects appear to be limited.

Comprehensive analysis of common SNPs at 22 circadian genes: associations with bipolar I disorder at NPAS2.
H. Mansour, M. Talkowski, J. Wood, K. Chowdari, L. McClain, K. Prasad, D. Montrose, A. Fagiolini, E. Friedman, M. Allen, C. Bowden, J. Calabrese, R. El-Mallakh, S. Faraone, M. Fossey, L. Gyulai, J. Loftis, P. Hauser, T. Ketter, V. Nimgaonkar Department of Psychiatry, University of Pittsburgh School of Medicine, Pittsburgh, PA.

Objective: Prior reports suggest association of key circadian genes with bipolar I disorder (BP1), schizophrenia (SZ) and schizoaffective disorder (SZA). Our objectives were: 1. to comprehensively test association at representative common polymorphisms of validated circadian genes; 2. to explore association with Morningness/Eveningness (M/E), a quantitative trait that reflects circadian phase and replicably distinguishes BP1 cases from controls. METHODS: We assayed 279 publicly available tag SNPs at 22 circadian genes in a sample of 523 patients with BP1, 532 patients with SZ/SA, and 477 screened adult controls. The composite scale (CS), a measure of M/E was completed by a subset of participants (n = 128 controls, n = 146 SZ patients). RESULTS: Gene-based tests, but not individual SNP analyses yielded significant associations between NPAS2 and BP1 (Bonferroni corrected $p = 0.02$). Similar trends were observed with SZ/SZA (corrected $p < 0.1$). Exploratory analyses suggested associations between CS scores and SNPs from 11 genes (n = 25 SNPs), with NPAS2 SNPs outnumbering the other genes (n = 9 SNPs). SNP rs13025524 at NPAS2 was nominally associated with BP1 and SZ/SZA. CONCLUSION: Several lines of evidence suggest that NPAS2 is associated with disorders in the BP1/SZ spectrum, as well as a quantitative trait related to these disorders. Replicate studies using sufficiently powered samples, as well as functional analyses are warranted. Additional authors: Lauren B. Marangell, David J. Miklowitz, Andrew A. Nierenberg, Jayendra Patel, Gary S. Sachs, Pamela Sklar, Jordan W. Smoller, Nan Laird, Matcheri Keshavan, Michael E. Thase, David Axelson, Boris Birmaher, David Lewis, Tim Monk, Ellen Frank, David J. Kupfer, Bernie Devlin.

Profound deficits in pancreatic islet function lead to hormone deficiency in a deletion mouse model of Prader-Willi syndrome (PWS). *M. Stefan[1,2], R. A. Simmons[3], S. Bertera[1,2], M. Trucco[1,2], F. Esni[1,2], P. Drain[2], R. D. Nicholls[1,2]* 1) Children's Hospital of Pittsburgh, Pittsburgh, PA; 2) University of Pittsburgh, Pittsburgh, PA; 3) University of Pennsylvania, Philadelphia, PA.

Type 2 diabetes is considered a frequent outcome of obesity in PWS. Nevertheless, several studies have shown that patients with PWS have increased insulin resistance and decreased insulin secretion relative to the degree of obesity. The mechanisms underlying these phenomena are unknown. Genetic defects in PWS lead to loss of function of multiple imprinted, paternally-expressed loci encoding proteins and small RNAs with most predicted to regulate other RNAs suggesting that multiple pathways could be affected in PWS. In a transgenic deletion mouse model of PWS (TgPWS) with neonatal failure to thrive we identified exceptionally low to undetectable levels of plasma insulin and glucagon in fetal and neonatal life. Circulating hormone deficiencies were associated with disrupted islet architecture, reduced and cell mass and decreased insulin, C-peptide and glucagon pancreatic content at postnatal (P) day 1. As circulating levels of pancreatic hormones were out of proportion to decreased and cell mass we used an Ins-C-Timer fluorescent reporter that changes color with time after insulin synthesis to monitor secretion from cells in TgPWS pancreas at P1. We found impaired insulin release which may explain the severe hypoinsulinemia in TgPWS mice. Analysis of pancreatic global gene expression by microarray and quantitative QRT-PCR, and expression studies using purified islets, showed that mRNAs encoding all islet hormones and many proteins involved in secretion/exocytosis are increased by 4-7.5 fold relative to islet cell mass in P1 TgPWS mice. This may represent an islet-specific compensatory response to deficient plasma hormone levels and/or a primary effect of a missing PWS gene. In conclusion, profound functional and developmental pancreatic islet deficits are found in a PWS mouse model. Elucidation of the basis for global islet hormone secretory abnormalities in the PWS mouse model may identify a new regulatory pathway of insulin and other hormone secretion.

Eight-year clinical outcomes of enzyme replacement therapy in 884 children with type 1 Gaucher Disease. *H. C. Andersson[1], P. Kaplan[2], K. Kacena[3], J. Yee[3]* 1) Hayward Genetics Ctr, Tulane Univ Medical Ctr, New Orleans, LA; 2) Children's Hospital of Philadelphia, University of Pennsylvania School of Medicine, Philadelphia, PA; 3) Genzyme Corporation, Cambridge, MA.

OBJECTIVE: To analyze the clinical responses to enzyme replacement therapy (ERT) in a large international cohort of children with type 1 Gaucher disease (GD1). METHODS: Anonymized data from 884 children in the Gaucher Registry were analyzed for the effect of long-term ERT on hematological and visceral manifestations, linear growth and skeletal disease. Data analysis used mixed effects models and Kaplan-Meier curves (bone pain and bone crises). Median ERT dose was 75u/kg/4wks. RESULTS: The median height Z-score for the study population was -1.4 at baseline and 34% of patients were less than normal 5%ile height. After 8y of treatment, the median height and percentiles approximated that of the normal population. Anemia was present in over 50% at baseline and resolved in all patients after 8y of treatment. Over 50% of patients had platelet counts below 100,000/cu mm at baseline but over 95% had platelets above this level after 8y of treatment. Liver and spleen volumes decreased over 8y of treatment. Mean bone density Z-score was -0.34 at baseline, and normalized within 6.6y of treatment. In patients reporting bone crisis before treatment (17%), no bone crises were reported after 2y of ERT. Few (2.5%) patients without bone crises pre-ERT at baseline had a crisis after the start of treatment. CONCLUSIONS: Within 8y of ERT most clinical parameters studied became normal or near normal. The data provide pediatricians a measure of expected response in GD1 patients for individual clinical parameters relative to baseline and offer the first long-term outcomes for a large worldwide pediatric cohort.

Differential Bias in Genotype Calls between Plates due to the Effect of a Small Number of Lower DNA Quality and/or Contaminated Samples. *A. Pluzhnikov[1], J. E. Below[1], A. Tikhomirov[1], A. Konkashbaev[1], S. Roe[1], D. Nicolae[1,2], N. J. Cox[1]* 1) Medicine/Genetic Medicine, Univ Chicago, Chicago, IL; 2) Statistics, Univ Chicago, Chicago, IL.

Quality control (QC) issues in genotype calling are becoming increasingly important in genome wide association studies. We examined the effect of a small number of samples that had lower than average DNA concentration, DNA fragmentation, contamination, or other quality issues not detected by standard QC, on genotype calling of other samples in the study using data from the Genetics of Kidneys in Diabetes (GoKinD) collection of more than 1600 unrelated probands with Type I diabetes. All samples were typed using the Affymetrix Genome-Wide Human SNP Array 5.0 platform, and genotypes were called by plate using the Birdseed v.2 algorithm to minimize the amount of missing data. We detected 8 problematic samples displaying unusual patterns of relatedness and high levels of heterozygosity and showed that, for a number of markers, these samples cluster together, thus altering the allele calls for other samples on the plate and leading to significant differential bias in allele frequencies between plates that eventually resulted in false-positive associations in the GoKinD data. We discuss ways to detect this kind of differential bias between plates, and to correct it depending on the availability of the raw intensity (.cel) files.

Skeletal features, cardiovascular involvement and outcomes of patients with TGFBR2 mutation and comparison with unaffected patients and patients with FBN1 mutation. *D. Attias, C. Stheneur, L. Faivre, MA. Delrue, H. Plauchu, S. Lyonnet, M. Rio, C. Francannet, M. Le Merrer, C. Boileau, G. Jondeau* APHP, Consultation multidisciplinaire Marfan, Hopital Bichat Claude Bernard, Paris, France.

TGFBR2 mutation is a rare event leading to still incompletely characterized phenotypes. We report clinical features and outcome of 94 patients with known TGFBR2 mutation, belonging to 26 different families. They were compared with a large group of 243 patients with FBN1 mutations matched for age and sex. Patients with TGFBR2 mutations were 28,3 16,1 y.o.; 49M/45F. Common skeletal in the TGFBR2 group were joint hypermobility (73.3 %), pectus anomaly (43 %) and highly arched palate (48.6 %. According to Ghent criteria, 86.1 % (62/72) of patients with TGFBR2 mutation showed less than 3 major skeletal criterion and 31.9 % patients (23/72) showed no major skeletal criterion. Mitral valve prolapse was present in 26% vs 45% in the group of patients with FBN1 mutation ($p<0.001$) and no surgery for mitral valve was performed. Maximal aortic diameter was observed at the level of the sinuses of Valsalva for all patients. However, the degree of dilatation was very variable from one patient to another : 27 % of patients with TGFBR2 mutation were without significant aortic dilation including 3 patients above 55 y.o. 27 patients died before or during the study period; the mean age of death was 36,314,6 years (range 9 to 67) with ascending aortic dissection and sudden death as leading cause. There was no difference in the occurrence of ascending aorta surgery (for aneurysm or dissection) between the two groups but patients with TGFBR2 mutation had to undergo this surgery younger (3113 years vs. 3912 years, $p = 0.01$). Conclusions TGFBR2 mutation is associated with mild skeletal features, less frequent and less diffuse than in patients with FBN1 mutation. There was no mitral valve prolapse leading to qurgery in the TGFBR2 group. Aortic involvement is more severe as a mean than in patients with FBN1 mutations. However it is very variable: moderate or no aortic dilatation was observed in some elderly patients carrying the mutation.

Massively parallel paired-end transcript sequencing of primary malignant glioblastoma. *M. J. Clark[1], N. Homer[2], Z. Chen[1], B. O'Connor[1], B. Merriman[1], S. Nelson[1]* 1) Human Genetics, UCLA, Los Angeles, CA; 2) Computer Science, UCLA, Los Angeles, CA.

Glioblastoma multiforme (GBM) is the most deadly form of brain cancer, having a median survival time of less than a year. Abnormal transcripts resulting from aberrant splicing and translocation events are common features. With the advent of massively parallel paired-end sequencing, we are capable of visualizing these abnormal transcripts on a genome-wide basis, which can be performed in parallel with sequence mutation discovery. We have performed paired-end sequencing using the Solexa Genome Analyzer II on full-length cDNA prepared from eight primary GBMs from separate patients. Randomly sheared cDNA libraries with an average insert size of 200 bases were selected, and we attempted to sequence 50 bases from both ends. BFAST was used to align the reads to RefSeq and to the genome. This method is sensitive to detecting fusion transcripts, known alternative splicing events, and novel mRNA isoforms, but relies on reasonable expression level of the transcripts. Further, for more highly expressed genes, the aligned sequences can be searched for differences relative to the consensus sequence of the human genome. We show that homozygous mutations leading to premature stop codons in PTEN are detectable with this approach. Novel transcripts unique to glioblastomas are strong candidates as key role-players in tumorigenesis.

Meta-analysis of genetic association studies and adjustment for multiple testing when SNPs or traits are correlated. *K. N. Conneely[1], M. Boehnke[2]* 1) Department of Human Genetics, Emory University, Atlanta, GA; 2) Department of Biostatistics, University of Michigan, Ann Arbor, MI.

A recent wave of large-scale genetic association studies has led to a host of positive and promising genetic association results. In turn, validation of these results through testing in independent samples and the boost in statistical power from combining results across samples have led to an increased focus on meta-analysis. Prospective meta-analysis, in which several samples are genotyped and phenotyped with the intent to combine results, has become more feasible due to decreases in genotyping costs and the collection of increasingly large samples. This includes the popular and efficient two-stage analysis, in which the most significant SNPs or traits from an initial study are followed up in independent samples. Retrospective meta-analysis, which combines results from published studies, remains common as a method to summarize and clarify findings from previous studies which may lack precision and power due to small sample size.

Meta-analyses of genetic association studies based on multiple SNPs and traits are subject to the same multiple testing issues as single-sample studies, but it is often difficult to accurately adjust for the multiple tests. Procedures such as Bonferroni may control the type I error rate but will generally provide an overly harsh correction if SNPs or traits are correlated. Depending on study design, availability of individual-level data, and computational requirements, permutation testing may not be feasible in a meta-analysis framework. We present methods and software for adjusting for multiple correlated tests under several study designs commonly employed in meta-analyses of genetic association tests. Our methods are applicable to both prospective and retrospective meta-analyses, and the study designs include cases where the same SNPs are not genotyped in all studies due to missingness-at-random or two-stage design. Simulations show that these methods accurately control the rate of type I error and achieve improved power over multiple testing adjustments that do not account for correlation between SNPs or between traits.

Micro CT analysis of bone phenotypes: Thresholding for accurate density vs. trabecular geometry across different scan resolutions. *B. Dawson[2], T. Sibai[1], B. Lee[1,2]* 1) Dept. Mol & H Genetics, Baylor College Med, Houston, TX; 2) Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX.

High resolution MicroCT scanning is primarily used for geometric and densitometric quantification of small animal bone tissues. The analysis of these samples requires a "thresholding" step that differentiates bone from background or lower density material not intended for analysis. Thresholding for density measurements and trabecular geometric estimation (BV/TV) is classically performed separately. Objective: To assess the optimum threshold that best reflects both density and trabeculal architecture simultaneously and to examine the mathematical relationship held between density and threshold. A phantom of known density of 1051 mg HA/ccm was scanned alongside a murine femur at four different resolutions (12, 16,20 and 30 m). The phantom was scanned at different resolutions by using a single fixed size sample holder but altering the softwares scan resolution in order to reduce position variability among scans. For each threshold increment of 10 of threshold values (150-300) commonly used for trabecula geometry estimation, the density of the standard phantom was assed using the machines configured trabecular bone analysis algorithm. The mouse femur was included alongside the phantom to help determine the accuracy of trabecular geometry at the threshold that best reflects the true density of phantom. Results: At higher resolution scans (12, 16, and 20 m), the density measurement across the range of thresholds is linear with best estimation of true density at thresholds of 182, 206, 226 respectively. The relationship between threshold and density at 30 m resolution was less linear especially at thresholds below 240. At the optimum density threshold, higher scan resolution tended to overestimate and lower resolutions tended underestimate trabecular geometry Conclusion: The optimum threshold for density estimation was inversely proportional to scan resolution .The density optimized threshold at 16 m resolution best represented trabecular architecture. These results should be considered when critically evaluating bone density data on animal models reported in the literature.

Mutation analysis of the GIGYF2 (PARK11) gene in 55 families with parkinsonism. *B. Schuele, L. C. Sterling, D. Tewari, K. K. Johansen, J. W. Langston* Parkinson's Institute, Sunnyvale, CA.

Objective: To determine the mutation frequency in the Grb10-Interacting GYF Protein 2 (GIGFY2) gene in cases with parkinsonism and family history. Background: A mutation frequency of 4.8% (12/249) in the GIGYF2 gene has been reported in patients with familial Parkinsons disease (PD) from France and Italy. Methods: PD cases with at least one affected relative were enrolled in the study. Cases and healthy controls were ascertained through the outpatient clinic at the Parkinsons Institute and signed IRB approved informed consent. All 28 coding exons including exon-intron boundaries were sequenced bi-directionally in 55 familial cases. Samples tested negative for the LRRK2 p.G2019S mutation. Detected sequence changes were genotyped using restriction enzyme digestion of the PCR products in controls and relatives. Results: We detected four novel sequence changes that were not present in 378 control alleles. Two changes (2/55, 3.6 %) were missense mutations (p.D349E and p.S1035C). One change was a 3-bp duplication c.3063_3065dupGCA and one was a synonymous variant (c.1983AG). In addition, we found a known synonymous variant (c.1716 GT), which was not present in the control population. The two amino acid substitutions, p.D349E and p.S1035C, were found at highly conserved regions of the protein, however, bioinformatic predictions of the pathogenicity using SIFT showed tolerable scores of 0.59 and 0.06, respectively. The carrier of the p.D349E change had LOPD (age at onset 78) showing a recessive mode of inheritance with two additional affected siblings. The patient with the p.S1035C change had a phenotype of EOPD (age at onset 27) with slow progression, and five affected relatives showing an dominant inheritance pattern with reduced penetrance. Conclusion: We detected a similar mutation frequency in our families from North America as was reported in families from France and Italy. To conclude that these variants are disease-causing, we are in the process of ascertaining DNA samples from additional family members to test if the variants segregate within the family. We will perform functional assays of the detected variants to determine pathogenicity.

Breakpoint location in 22q11 deletions determined by aCGH. *D. C. Bittel[1], S. Yu[1], H. Newkirk[1], N. Kibiryeva[1], A. Holt III[1], M. G. Butler[2], L. Cooley[1]* 1) Section of Medical Genetics, Children's Mercy Hospital, Kansas City, MO; 2) 6410 Hillside St. Shawnee, KS 66218.

A hemizygous deletion of chromosome 22q11 commonly results in conotruncal cardiac defects along with a variable group of other anomalies. Four distinct highly homologous blocks of low copy number repeats (LCRs) flank the deleted region. Mispairing of the LCRs during meiosis resulting in unequal meiotic exchange is hypothesized to be the mechanism causing the deletion. The proximal LCR is located on 22q11 from 17.037 to 17.083 Mb and the distal LCR is located from 19.835 Mb to 19.880. Although the breakpoints are known to localize to the LCRs, the position of the breakpoints have not been investigated in more than a few individuals. We used high resolution oligo-based array Comparative Genomic Hybridization (aCGH) to resolve the breakpoints in 20 subjects with 22q11 deletions. We also investigated copy number variation (CNV) in the rest of the genome. It appears that the 22q11 break can occur on either side of the LCR, although more commonly on the distal side of the first LCR. The proximal breakpoints in our subjects spanned the region from 16.932 Mb to 17.343 Mb. This region includes the genes PEX26, (peroxisome biogenesis factor 26); TUBA8, (tubulin, alpha 8) USP18, (ubiquitin specific protease 18); DGCR6, (DiGeorge syndrome critical region protein 6); PRODH, (proline dehydrogenase 1) along with several open reading frames that may encode proteins of unknown function. The distal breakpoints in our subjects spanned the region from 19.788 Mb to 20.136 Mb. This region includes the genes GGT2, (gamma-glutamyltransferase-like protein 2); HIC2, (hypermethylated in cancer 2) and multiple transcripts of unknown function. The genes in these two regions are variably hemizygous depending on the location of the breakpoint. These 20 subjects had 75 CNVs, 15 duplications and 60 deletions ranging in size from 1 Kb to 10 Mb. The variable presence or absence of genes at the breakpoints plus the variation in the genome due to CNVs likely contributes to the variable phenotype associated with DiGeorge/velocardiofacial syndrome.

Identification of a Single Gene Locus for Canine Squamous Cell Carcinoma of the Digit. *E. A. Ostrander[1], B. vonHoldt[2], E. Karlins[1], D. Mosher[1], J. Mulliken[1,3], H. Parker[1], R. K. Wayne[2], D. M. Karyadi[1]* 1) NHGRI, NIH, Bethesda, MD; 2) Dept of Biology, UCLA, Los Angeles, CA; 3) NISC, NIH, Bethesda, MD.

Squamous cell carcinoma (SCC) of the digit is a highly breed-specific skin cancer with increased risk found in large black dogs, including the standard poodle, giant schnauzer, briard and Gordon setter. This cancer is more aggressive than most SCCs with 80% of cases involving bone lysis and 5-13% recurring in multiple toes. When invasive, metastasis to the lung or lymph nodes ultimately leads to death. Using the canine-specific Affymetrix chip, containing 127,000 single nucleotide polymorphisms (SNPs), we conducted a whole genome association study for SCC of the digit using 31 standard poodle cases and 34 controls. SNPs were removed from the analysis if greater than 10% of the data was missing, there were more then 60% heterozygous calls, or minor allele frequency was less than 1%. The final SNP set consisted of 45,078 SNPs. Using the single-locus chi-squared test of significance, we calculated the allelic association of each SNP with the disease phenotype. The top 6 most significant SNPs were all at the same genomic locus (P_{raw}=5.62x10^{-5}-1.20x10^{-7}). Chromosome-wide permutations (n=100,000) were performed to test for significance yielding a chromosome-wide empirical value of P=7.00x10^{-5} for the most significant SNP. Five other SNPs in this region were also significant (P_{emp}=0.0299-0.0010). No other genomic regions yielded peaks significant at the chromosome-wide level. Initially, the distance spanned by the significant peak was 999.4 kb, and included four known and four predicted genes. After additional SNP genotyping and sequencing haplotype analysis, utilizing over 325 markers, resolved the region to 812.2 kb with 3 crossovers on each side. A reduced region, defined by 1 crossover, spans 515.7 kb, and contains only 1 complete gene plus 2 exons of a second gene. The complete gene is an excellent candidate and is a potential oncogene. The entire region is being scanned using a capture technology combined with Solexa sequencing to identify the causal variant. Identification of the mutation underlying this canine cancer is likely to inform human forms of SCC.

Inside the brain in Angelman Syndrome: Phenotypic characterization using advanced neuroimaging techniques.

S. U. Peters[1], C. A. Bacino[2], Z. Chu[3], R. Yallampalli[4], J. V. Hunter[3], E. A. Wilde[4] 1) Dept Pediatrics, Baylor Col Medicine, Houston, TX; 2) Dept. Molecular & Human Genetics, Baylor Col Medicine, Houston, TX; 3) Dept. Radiology, Baylor Col Medicine, Houston, TX; 4) Dept. Physical Medicine & Rehab, Baylor Col Medicine, Houston, TX.

Angelman syndrome (AS) is a neurogenetic disorder that is characterized by severe mental retardation, lack of speech, ataxia, seizures, and frequent outbursts of laughter. We utilized quantitative magnetic resonance imaging (MRI) and diffusion tensor imaging (DTI) techniques to examine the neuroanatomical correlates and white matter alterations that contribute to the phenotype in AS. Here we present preliminary findings of five deletion positive patients with AS and five age and gender matched controls. Results of DTI studies reveal significant differences between AS patients and controls for white matter pathways involving the frontal, temporal, parietal, and limbic areas. Patients with AS exhibited lower fractional anisotropy values and higher apparent diffusion coefficient and radial diffusivity values in these regions. Significant correlations were noted between cognitive, language, motor, and memory skills and impairment in frontal regions. Abnormalities in temporal pathways were associated with fine motor and expressive language deficits. Abnormalities in limbic pathways were correlated with deficits in socialization and play skills. Results of quantitative MRI indicate that after controlling for total intracranial volume, AS patients have reduced white matter volumes in the cerebellum and cerebrum. Reduced volumes were also noted for the hippocampus, caudate, and putamen relative to controls. Reduction in volume in the cerebellum, caudate, and putamen was significantly correlated with gross motor abilities while impairments in play and social skills were significantly correlated with amygdala volume. These findings demonstrate that AS patients exhibit microstructural changes in white matter fiber tracts that affect the development, wiring, and targeting of axons that link affected brain regions. They also exhibit reduced volume in brain regions that directly contribute to the phenotype observed.

What's in an aging clinic? Family concerns and medical issues in Cornelia de Lange syndrome. *A. D. Kline[1], J. Peracchio[2], M. Simonson[3], A. Kimball[1]* 1) Harvey Inst Human Gen, Greater Baltimore Medical Ctr, Baltimore, MD; 2) Cornelia de Lange Syndrome Foundation, Avon, CT; 3) Johns Hopkins Univ/National Human Genome Research Inst, NIH Genetic Counseling Graduate Program, Bethesda, MD.

Multidisciplinary clinics for the assessment of aging are rarely found for specific genetic syndromes. We have held such a clinic for older individuals with Cornelia de Lange syndrome (CdLS) for seven years. A total of 59 patients have been seen with an equal male to female ratio, and age range from 11 to 50 years, with 36% over 21 years. Of these, 47% are severely involved, 19% moderately and 34% mildly. Parent(s) accompanied 98% of the patients, and 22% brought siblings, with 42% from outside our mid-Atlantic region. Seventy-five percent live with the family and 3% have died. The diagnosis of CdLS was questioned in three additional patients. Regarding family concerns, the greatest have been behavioral, especially in the moderately affected group, and then gastrointestinal (GI), although lower than expected for the high incidence of GI involvement. Other concerns have included nutrition and weight, regardless of age or severity, dental, particularly in the most severe group, orthopedic issues in those under 21 years, and reproductive health issues in females. Medical findings and evidence for premature aging from this clinic have been previously reported [Kline et al., AJMG 145C:248-60, 2007]. Recommendations were GI-related in 68% of patients, behavioral in 53%, with medication adjustments in 31%, and related to diet and exercise in 34%. Preventative diagnostic work-up was recommended in 36%. Other recommendations included hormonal testing, dental work, and audiology evaluation. State funding, work-related issues after schooling, and other social needs were also raised. Clearly, there is a need for additional regional clinics, as well as specific diagnostic studies recommended on all diagnosed patients. A major component of aging is the existing concerns by siblings related both to future care of the affected individual and recurrence. Assessment of the genetics of healthy aging is a recent public health initiative and similar clinics could elucidate some important findings.

Rai1 overexpression in specific brain regions is enough to induce phenotypes observed in PTLS mouse model. *J. Molina, P. Carmona-Mora, J. Young, K. Walz* Centro de Estudios Científicos, Valdivia, Valdivia, Chile.

Retinoic acid induced 1 gene (RAI1) is a dosage-sensitive gene located within the human chromosome 17p11.2 region. Mutations in RAI1 have been associated with Smith-Magenis syndrome (SMS; MIM182290). Duplication of 17p11.2 chromosome region, results in the Potocki-Lupski syndrome (PTLS; MIM610883) characterized by mental retardation, growth delay, reduced body weight, anxiety, hyperactivity and autistic behaviors. The objective of this work is to study when and where overexpression of Rai1 is causative of the PTLS phenotype. For this purpose we generated mice that bear an inducible wild-type Rai1 allele in a wild type genetic background. We tagged a wild type copy of Rai1 cDNA with HA and subcloned it downstream of a bidirectional tetracycline-responsive promoter (EGFP-pBI-Rai1-HA) to generate transgenic mice that bear an extra copy of Rai1. We crossed the Rai1 transgenic mice with those expressing tTA under CamKII or Eno2 promoter regulation. In these EGFP-Pbi-Rai1-HA; Eno (or CamKII)-tTA transgenic mice, expression of the Rai1 transgene can be initiated in a spatial promoter-dependent manner at different stages of development. Brain sections analysis of double transgenic mice, EGFP-Pbi-Rai1-HA, CamK2-tTA or EGFP-Pbi-Rai1-HA, Eno-tTA showed the expected EGFP And Rai1-HA pattern. In a first and rapid screening in order to see if the overexpression of Rai1 in different brain regions was enough to trigger a phenotype present in PTLS mouse model (Dp(11)17/+) we weighted the double transgenic mice from the first to the sixth month of life. Dp(11)17/+ mice are significantly underweight when compared to their wild type littermates. Interestingly enough we found that mice overexpressing Rai1 in different brain regions are significantly slimmer that their wild types littermates. Moreover, in Dp(11)17/+ mice the differences of weight are due to a decrease in adipose tissue when compared to wild type littermates a similar phenotype was found when we analyze these double transgenic mice. These results indicate that overexpression of Rai1 in specific brain regions is enough to trigger some of the phenotypes observed in PTLS mouse model.

Keratitis-ichthyosis-deafness (KID) syndrome: a connexin 26 gap junction disorder. *G. Oswald[1,5], K. Puttgen[2,5], B. Cohen[2,5], T. Wakefield[3,5], G. Raymond[4,5,6], J. Hoover-Fong[1,5]* 1) McKusick-Nathans Institute of Genetic Medicine; 2) Dept of Pediatric Dermatology; 3) Dept of Pediatric Otolaryngology; 4) Dept of Pediatric Neurology; 5) Johns Hopkins Univ, Baltimore, MD; 6) Kennedy-Krieger Institute, Baltimore, MD.

KID syndrome (MIM 148210) is a rare, congenital AD form of ectodermal dysplasia characterized by Keratitis, Ichthyosis and Sensorineural Deafness. It is caused by heterozygous mutations in gap junction beta 2 (GJB2) encoding connexin 26 (*Cx26*). Mutations in GJB2 also cause AD and AR nonsyndromic hearing loss, DFNA3 and DFNB1 respectively, and other AD keratoses with/out SNHL (e.g. hystrix-like ichthyosis-deafness, palmoplantar keratoderma, Vohwinkel syndrome).

A 10-month old Caucasian male was admitted for severe failure to thrive after foster care placement. Limited medical information revealed ectodermal dysplasia diagnosed at 2 months of age and failed hearing screens. Current findings include diffuse ichthyotic skin, photophobia, dystrophic nails, sparse eyebrows, eyelashes and hair, plagiocephaly, epidermal cyst and hypertonia; he could tear and sweat. Audiogram confirmed deafness and KID syndrome was proposed.

Molecular analysis of Cx26 revealed a heterozygous GA transition at codon 50 causing substitution of aspartic acid with asparagine, the most common mutation reported to cause KID syndrome. Most cases represent sporadic dominant mutations, though gonadal mosaicism or decreased penetrance has been suggested. Other complications of KID syndrome include inflammatory nodules, squamous cell carcinoma, epidermal cysts and corneal vascularization abnormalities.

Skin improved with aggressive lubrication and topical steroids, though abscesses of the neck and head have occurred. Photophobia has resolved and vigilant ophthalmologic followup is ongoing plus evaluation for cochlear implants. Early nutritional and psychosocial deficits likely contributed to poor weight gain and plagiocephly and are likely not part of KID disease spectrum.

TGF- and BMP- Signaling Pathways have Antagonistic Effects during Chondrogenesis. *B. Keller[1], T. Bertin[1], Y. Chen[1], E. Munivez[1], B. Dawson[2], P. Hermanns[3], B. Zabel[3], B. Lee[1,2]* 1) Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX; 2) Howard Hughes Medical Institute, Baylor Col Medicine, Houston, TX; 3) Centre for Pediatrics and Adolescent Medicine, University Hospital of Freiburg, Germany.

TGF/BMP signaling is essential for the development of the majority of embryonic and extraembryonic tissues. Ligands of these pathways are involved in the development of cartilage and bone at multiple stages. *Smad1* and *Smad5* are mediators of the BMP signals and both are expressed in the growth plate. TGF signaling has been shown to exhibit diverse effects both *in vitro* and *in vivo*. To compare the role of these signaling pathways in the growth plate, we generated mouse models of loss of BMP vs. TGF signaling function. To down regulate BMP signaling in vivo, we generated chondrocyte-specific deletions of *Smad1* on either a wild type and *Smad5+/-* background. Conditional deletion of *Smad1* in proliferating chondrocytes results in a slight shortening of the growth plate, while an additional *Smad5+/-* deletion leads to a more severe phenotype with shorter prehypertrophic and hypertrophic zones and a decreased proliferation rate. *Ihh* expression is down-regulated. Morphologically, the conditional *Smad1* deletion with and without *Smad5+/-* background leads to craniofacial alternations characterized by agenesis of the nasal septum. To down regulate TGF signaling in chondrocytes, we generated transgenic mice over-expressing a dominant negative form of the TGF receptor I (TRI). These mice are smaller but show histologically an elongated growth plate with enhanced *Ihh* expression, an increased proliferation rate and disturbed production of the extracellular matrix. Morphologically, they also exhibited a craniofacial phenotype: smaller skull with a prominent mandible. Hence, during endochondral ossification, BMP and TGF signaling have antagonistic effects on cell proliferation and differentiation. While disruption of BMP signaling leads to a shortening of the growth plate with fewer proliferating cells, impairment of TGF signaling results in an increased proliferation rate and thus a longer prehypertrophic and hypertrophic zone.

SNPs associated with normal variation in adult human height are not associated with osteoarthritis susceptibility.

K. Chapman[1], E. Zeggini[1], A. Gonzalez[4], N. W. Rayner[1], E. Suchiman[2], M. Kloppenburg[2], J. Rodriquez-Lopez[4], M. N. Weedon[5], T. M. Frayling[5], P. E. Slagboom[2], J. Loughlin[3], I. Meulenbelt[2] 1) WTCHG, Oxford, UK; 2) Leiden, The Netherlands; 3) Newcastle upon Tyne, UK; 4) Santiago, Spain; 5) Exeter, UK.

Osteoarthritis (OA) is a late-onset complex disease. A compelling genetic association with OA of a functional SNP (rs143383) in the 5' UTR of the *GDF5* gene has been observed in Asian and European cohorts. Intriguingly, this SNP is also significantly associated with normal variation in human height, opening up the possibility that there are common mechanistic pathways between these two polygenic traits. A large number of additional loci that influence human height have recently been reported. Our aim was to assess whether these SNPs were also associated with risk for primary OA. We genotyped 25 SNPs, previously associated with normal variation in human height, in a total of 4901 case-control samples. These were ascertained from 3 European cohorts in Spain (810 cases, 294 controls), the UK (1854 cases, 833 controls) and the Netherlands (191 sib-pairs from the GARP study, 728 controls). Association with overall OA was examined in the 3 populations individually and by meta-analysis using a fixed-effect model. Two SNPs were associated with OA in the Spanish cohort; rs16896068 within *LCORL* (OR 1.42, 95% CI 1.10-1.82), p=0.006 and rs1390401 within *ZNF678* (OR 1.64, 95% CI 1.23-2.18), p=0.0009. However, they were not replicated in the GARP (p=0.06 and p=0.48 respectively) or UK (p=0.71 and p=0.78 respectively) cohorts. Meta-analysis provided no evidence for significant association with the lowest p-value for rs9289476 within *ANAPC13* (combined OR 0.9, 95% CI 0.82-0.99), p=0.04. This was also the strongest signal in the UK sample (p=0.07). In conclusion, we do not find robust evidence for association to overall OA of any of the 25 SNPs genotyped. Within the power limits of our study we will further explore stratified associations to specific OA subtypes and/or gender. Our study indicates that, if human height and OA have a shared genetic component, the effect sizes of common variants affecting both traits are likely to be small.

Evidence of Genetic Epistasis and Sex-Gene Interactions in Susceptibility to Hypertension. *P. A. Shih[1], J. H. Moore[2], B. K. Rana[1], M. Mahata[1], S. Mahata[1], D. T. O'Connor[1]* 1) Department of Medicine, University of California, San Diego, San Diego, CA; 2) Computational Genetics Laboratory, Dartmouth Medical School, Lebanon, NH.

Hypertension is a multi-factorial disease. Here we applied case-control associations to explore high-order gene-gene and gene-sex interactions among 82 polymorphisms (SNPs) in three biological pathways known to affect hypertension risk. A cross-sectional study was analyzed using 679 hypertensive cases (352 male/327 female, mean SBP=154/DBP=99) and 704 normotensive controls (305 male/399 female, mean SBP=108/DBP=56) from a large, community-based sample. TuRF was used to filter 82 SNPs and to select a subset of 10 contributors most likely to interact. Epistatic interactions were probed using Multifactor Dimensionality Reduction (MDR). Association tests were conducted using Fisher's exact test and ANOVA. In vitro study of IL6 on *CHGA* promoter activity was conducted by luciferase assay. Of four candidate SNPs contributing to epistatic effects, *CHGA (3UTR)* , *IL6*, and *COMT* revealed individual associations with BP status ($p < 0.005$). Gene-sex interaction effect on risk was observed initially in MDR, while further association tests showed differential SNP-DBP associations for *CHGA (3UTR)* , *IL6* , and *COMT* polymorphisms, when analyzing men and women separately. *CHGA (3UTR)* was associated with DBP in men ($p = 0.004$) but not in women, while *COMT* was associated with DBP in women ($p = 0.0002$) but not in men. *IL6* was associated with DBP both in men and women. Multivariate ANOVA further showed evidence of epistasis on risk for 6 sets of interactions. Luciferase analysis revealed increased transcription of *CHGA* promoter upon stimulation by IL6. This study revealed association of not only individual SNPs with risk of hypertension, but also sex-gene and gene-gene interactions contributing to risk. Epistasis between inflammatory and adrenergic pathways was further confirmed in an in vitro assay, suggesting a potential novel mechanism for hypertension risk.

Mutation spectra in *PITPNM3* known as a cause of autosomal dominant cone rod dystrophy (CORD5). *L. Kohn[1], S. Haraldsson[1], S. Kohl[2], C. F. Inglehearn[3], O. Sandgren[4], I. Golovleva[1]* 1) Medical Biosciences/Medical and Clinical Genetics, Umeå University, Sweden; 2) Centre for Ophthalmology, Institute for Ophthalmic Research, Molecular Genetics Laboratory, Tübingen, Germany; 3) Section of Ophthalmology and Neuroscience, Leeds Institute of Molecular Medicine, St. Jamess University Hospital, Leeds, United Kingdom; 4) Clinical Biosciences/Ophthalmology, Umeå University, Sweden.

Autosomal dominant cone dystrophy (CORD5) is a rare disease predominantly affecting the cone photoreceptor cells. The main clinical symptoms are impaired visual acuity, sensitivity for light and defective colour vision. The disease locus was initially mapped to chromosome 17p13 and later on narrowed down from 27 cM to 14.3 cM exploring material from two Swedish families (Balciuniene et al, 1995; Köhn et al, 2007). Sequencing of a candidate gene, phosphatidylinositol transfer membrane-associated protein (*PITPNM3*) revealed a missense mutation, Q626H. *PITPNM3* known as a human homolog of the *Drosophila* retinal degeneration B (*rdgB*) gene is needed for transport of phospholipids, renewal of photoreceptors membrane and provides the ERG response to light. The plausible mutation causing CORD5 is located in the C-terminal region interacting with a member of nonreceptor protein tyrosine kinases, PYK2. Through collaboration with research groups in Germany and UK we have ascertained DNA from cone and cone-rod dystrophy patients for further screening of *PITPNM3* to establish the global impact of this gene in retinal diseases. Mutation screening of *PITPNM3* was performed by dHPLC (WAVE, Transgenomic) followed by DNA-sequencing. 111 samples were analysed in total. Several potential pathogenic mutations located in both coding and intronic sequences were found in the trial. Matched control populations are currently screened to elucidate whether or not these sequence variations are true mutations. In conclusion, the findings of additional mutations to Q626H in PITPNM3 will provide stronger evidence for novel pathways and a potential important role of PITPNM3 in mammalian phototransduction and molecular pathogenesis of retinal degenerations.

Comprehensive analysis of 40 kb in the 19p13 region points to a novel risk variant predisposing to celiac disease.

G. Trynka[1], A. Zhernakova[2], J. Romanos[2], L. Franke[1,2], K. A. Hunt[3], G. Turner[4], A. Ryan[4], D. Barisani[5], R. McManus[4], D. A. van Heel[3], C. Wijmenga[1,2] 1) Genetics Dept. UMC Groningen, The Netherlands; 2) Complex Genetics, UMC Utrecht, The Netherlands; 3) Barts and the London, School of Medicine and Dentistry, UK; 4) Dept. Clinical Medicine, Institute of Molecular Medicine, Trinity College Dublin, Ireland; 5) Dept. Experimental Medicine, University of Milano Bicocca, Italy.

Myosin IXB (MYO9B, 19p13) is one of the first non-HLA genes to be associated to celiac diseases (CD), an autoimmune disorder characterized by inflammation of the small intestine in response to gluten. MYO9B variants have also been shown to predispose to other traits (ulcerative colitis, type 1 diabetes and dermatitis herpetiformis), suggesting that it is a general risk factor in autoimmune disorders. In this study we have selected 20 individuals for sequence analysis of the entire associated region of MYO9B (30-kb LD block) and the two downstream genes, USE1 (6.6kb) and OCEL1 (4kb), which are in strong LD with the associated block (D>0.8). We have identified 165 sequence variants, of which 34 were novel. We further genotyped 67 of these SNPs in extended case-control cohorts in 3 populations from the Netherlands, Ireland and the UK (total of 1682 cases, 3258 controls). In the combined analysis we observed the strongest association for rs388484 (p=1.2E-4). This variant is located in the first intron of the USE1 gene, the hematopoietic stem/progenitor cells protein that also plays a role in the vesicle transport. We are now typing an Italian cohort to further validate this finding and are performing haplotype analysis to narrow down the association. In addition, we will include imputed genotypes from the previously performed genome-wide association study. Conclusions: By sequencing and further fine-mapping of the MYO9B associated block in multiple CD cohorts, we have replicated the association to the 19p13 block and may have located the true signal to the USE1 gene. Our study proves that deep sequencing of the associated locus with further replication of all variants from the region is a good strategy for fine-mapping.

Allopregnanolone in cyclodextrin significantly effects hepatic disease in feline Niemann-Pick type C. *C. Vite[1], W. Ding[1], C. Bryan[1], P. O'Donnell[1], M. Haskins[1], T. van Winkle[1], S. Walkley[2], S. Mellon[3], M. Vanier[4]* 1) School of Veterinary Medicine, University of PA; 2) Albert Einstein College of Medicine, Yeshiva University; 3) University of California, San Francisco; 4) INSERM Unit 820, Lyon-Laennec Medical School, Lyon, France.

Subcutaneous injections of 25 mg/kg of the neurosteroid allopregnanolone in hydroxypropyl-beta-cyclodextrin (Allo/HPBCD) were given to two cohorts of ages of cats with a mutation in NPC1 (C955S) and with clinical signs of Niemann-Pick type C (NP-C) disease: six cats were treated at 1, 3, 7, 14, and 21 days of age (Allo/HPBCD-Early) and six cats were treated weekly from 3 to 11 weeks of age (Allo/HPBCD-Late). Allo/HPBCD-Early cats showed no measurable benefit compared to untreated cats. In contrast, Allo/HPBCD-Late cats were significantly different ($p<0.05$) from untreated cats by the following measures: weighed more from 5 weeks of age until death, less serum alanine aminotransferse and aspartate aminotransferase activities, greater serum albumin, less serum cholesterol, and less serum and CSF chitotriosidase activity. In liver tissue, cholesterol, sphingomyelin and free sphingosine concentrations were also significantly less. Allo/HPBCD-Late cats were NOT significantly different from untreated cats by any measure of neurological dysfunction. Why was Allo/HPBCD ineffective at treating neurological disease in the cat when it effectively treated the npc1-/- mouse? Several possibilities exist: 1) Allopregnanolone may not cross the blood brain barrier sufficiently in the cat. 2) Allopregnanolone may not activate pregnane X receptor-dependent pathways as they do in the mouse. 3) Allopregnanolone may require in utero administration in the cat in order to mimic its effects at P7 in the mouse. 4) Finally, the failure of Allo/HPBCD to treat neurological disease in feline NP-C disease may be due to the inability of adequate levels of HPBCD to reach the brain by crossing an intact blood-brain barrier. Based on data in the mouse, we hypothesize that increasing CNS concentrations of HPBCD will improve the storage in the brains of cats and that improvement in neurological disease will be seen.

Amniotic Band Syndrome. A case report with bilateral choanal atresia. *A. Del Toro-Valero[1,2], N. Davalos-Rodriguez[1,3], G. Hernandez-Zaragoza[1], S. Barrios-Guyot[3], V. Vargas[3], A. L. Plascencia-Rocha[3], A. Estrada-De la Fuente[3], R. E'Vega-Hernandez[1,2], M. G. Lopez-Cardona[1,3]* 1) Instituto de Genetica Humana, Universidad de Guadalajara, Guadalajara, Jalisco, Mexico; 2) Becario CONACYT; 3) Hospital Regional Dr. Valentin Gomez Farias del ISSSTE, Jalisco, Mexico.

Del Toro-Valero Azucena1,2, Dávalos-Rodríguez Nory1,3, Herrnández-Zaragoza Guillermo2, Barrios-Guyot Selene3, Vargas Víctor3, Plascencia-Rocha Ana Lilia3, Estrada-De la Fuente Alejandro3, EVega-Hernández Ricardo1,2, López-Cardona María Guadalupe1,3. 1.-Instituto de Genética Humana, Universidad de Guadalajara. 2.-Becario CONACyT 3.-Hospital Regional Dr. Valentin Gomez Farias del ISSSTE,Jalisco,Mexico. Introduction: The Constricting Bands Syndrome (CBS) is a group of congenital malformations that include constricting bands and lymphadema, tumecaction and amputacion of fingers, arms and legs, facial asymmetry, encephalocele, anencephalia, pseudosyndactyly and microphtalmos. The prevalence rate is 7.7/10,000. Etiology is still unknown. It has been associated with preterm delivery, low birthweight and oligohydramnios. Exist two theories (endogenous and exogenous) proposed in the pathogenesis that can explain the most, but not all of the features. so exist a third theory called vascular, that can explain the intravisceral anomalies of the syndrome. Case report: Propositus is a male of 45 days old, product of III pregnancy of healthy and non-consanguineous parents. Throughout the pregnancy, the mother show frequent cough with sputum. The product was obtained by caesarean at 36 weeks in duration with 2800gr weight. Was present a few dark bands around their hands and feet, encephalocele, hydrocephaly, decrease in the size of the right ventricle, hypoplasia of the corpus callosum, absence of superior portion of parietal, frontal and occipital skull, facial asymmetry, bilateral choanal atresia, hypoplasia of the right ocular globe, distal tumefaction of limbs, absence of some phalanges and amputation of some fingers. Discussion: The purpose of this report is to present the case of a patient diagnosed with CBS which also present bilateral choanal atresia, not described above, that can be consequence of the syndrome.

Overexpression of Trpc6 in mouse podocytes causes kidney disease. *CP. Canales[1], P. Krall[1,2], K. Walz[1]* 1) Centro de Estudios Científicos, Valdivia, Chile; 2) Universidad Austral de Chile, Valdivia, Chile.

The TRPC6 channel (Transient receptor potential channel 6) is a member of the TRPC family that presents six transmembrane domains. It is activated by Di-acilglicerol (DAG) and is related with the calcium transport towards the cytoplasm through a pore located between the transmembrane domains 5 and 6. Since 2005, mutations in the TRPC6 gene have been associated to patients with Focal and Segmental Glomerulosclerosis (FSGS), pathology with dominant autosomic inheritance. In kidney, TRPC6 is enriched in the collecting ducts and the podocyte foot processes, where it co-localizes with podocin and nephrin. In 2006, it has been demonstrated that elevated levels of wild-type TRPC6 protein, in a transient in vivo over expression model, lead to podocyte dysfunction evidenced by the appearance of proteinuria. Taken together, these data suggest that TRPC6 play a key role in the pathogenesis of FSGS. With the hypothesis that the over expression of wild-type TRPC6 seems to be sufficient to cause proteinuria in healthy mice, our goal was the generation of a transgenic mouse that over express the wild type form of Trpc6 in the podocytes and its subsequent phenotypic characterization. We have generated a construct with the full length Trpc6 cDNA and we have added a hemaglutinin (HA) tag. This construct was subcloned into an expression vector, downstream of the human podocin promoter (pNPHS2) to direct expression to the podocytes. After in vitro studies, the construct was microinjected to generate transgenic mice. Three independent lines with different copy number integration and expression levels of the transgene were analyzed. Phenotypic characterization of the lines showed a significant increased proteinuria in 40-50% of the mice, with different onset ages that ranked between 5-10 months of age and abnormal urine protein pattern by SDS-PAGE analysis. Abnormal glomerular morphology was also observed by H&E staining. Finally, a podocyte specific marker has been detected in 24h urines samples of transgenic proteinuric mice. We can conclude that overexpression of Trpc6 in a podocyte specific manner gives a kidney disease that may be related to podocytes loss.

Expanding the Clinical and Molecular Spectrum of Timothy Syndrome. *T. A. Maher[1], G. Zhou[1], J. M. Milunsky[1,2]*
1) Center for Human Genetics; 2) Departments of Pediatrics, Genetics and Genomics, BUSM, Boston, MA.

Timothy syndrome (TS; MIM 601005)is a multi-system disorder characterized by lethal arrhythmias (prolonged QTc), cutaneous syndactyly of the fingers and toes (2/3), congenital heart defects, facial dysmorphism, hypotonia, seizures, autistic spectrum disorder (ASD), and mental retardation (MR). This rare disorder has been reported to result from an identical, *de novo* missense mutation (G406R) in the alternately spliced exon 8A of the *CACNA1C* gene. One additional mutation (G402S) has also been reported in exon 8 of the gene and typically results in a longer QTc without syndactyly. Expression studies have shown that the G406R mutation produces maintained inward calcium currents by causing nearly complete loss of voltage-dependent channel inactivation. We report a 3½ year old Hispanic male who meets the cardinal diagnostic features of TS including a prolonged QTc (up to .546), characteristic facies, hypotonia, bilateral 2/3 toe syndactyly, congenital heart disease (VSD and PDA), seizures, MR, and ASD. No MRI data has been reported on individuals diagnosed with TS. MRI of our patient revealed diffuse pachygyria and microcephaly. Sequencing of exon 8/8A of the *CACNA1C* gene was normal. However, full sequencing of the gene revealed a *de novo* missense mutation (G419R) in exon 9. G419R was not found in 300 normal controls or in the SNP database, and was predicted to be not tolerated by SIFT. G419R is in the same functional linker domain as the major mutation (G406R) known to cause this syndrome. Several genes that result in neuronal migration disorders (*Lis1* and *DCX*) when mutated, function through calcium dependent signaling. This may offer a clue to the underlying pathophysiology behind the pachygyria seen in our TS patient. We report for the first time a novel missense mutation not involving Exon 8/8A of the *CACNA1C* gene that leads to TS. We also expand the clinical phenotype to include pachygyria that may explain the MR, seizure disorder, and ASD in TS patients. Further neuroimaging studies are recommended in other TS patients. An EKG should be considered in patients with pachygyria and 2/3 toe syndactyly to evaluate for a prolonged QTc.

Clinical Molecular Genetic Testing for Primary Ciliary Dyskinesia. *M. Zariwala, K. Chao, M. Langley, M. Leigh, J. Booker, M. Knowles, K. Weck* Dept Path/Lab Medicine, Medicine, pediatrics, UNC School of Medicine, Chapel Hill, NC.

Primary ciliary dyskinesia (PCD) is a rare, autosomal recessive disorder due to impaired mucociliary clearance associated with abnormal ciliary structure and function. It is associated with oto-sinu-pulmonary disease, male infertility, and in some cases situs inversus, termed Kartagener syndrome. Diagnosis of PCD relies upon clinical phenotype, ultrastructural studies of cilia, nasal nitric oxide levels, and ciliary beat frequency. Current diagnostic methods are challenging and cumbersome. Development of a molecular genetic test panel was predicted to aid with diagnosis in some patients. Mutations in two ciliary outer dynein arm genes (*DNAI1* and *DNAH5*) have been noted in ~38% of well characterized PCD patients. Clinical molecular genetic testing using sequence analysis of selected exons with mutation clusters is available at the UNC Molecular Genetics Laboratory. Sequencing of the complete coding region of *DNAI1* is also performed for patients with one mutation identified. Since May 2007, 41 patients have been referred for PCD genetic testing and mutations are found in four on at least one allele. Patient #3372 had Cri-cu-chat syndrome, and features of PCD with a monoallelic deletion of chromosome 5p, which includes *DNAH5*. We detected a known *DNAH5* mutation [F476SfsX26] on the other allele, thus confirming the diagnosis of PCD. One patient (#1896) had a known founder mutation [IVS1+2_3insT] and a novel missense variant [R124C] in *DNAI1*. R124C was also found in another unrelated PCD patient (#1456) and was not detected in 196 control chromosomes. This patient had a splice mutation on the other allele and both these variations were also present in an affected sibling. A PCD patient (#1457) had 2 novel variants of unknown significance [I567T+F451N] in trans in *DNAI1*; both these residues are evolutionarily conserved and not present in 180 control chromosomes. In patient #2361, known *DNAH5* mutation [I1855NfsX6] was found on one allele. We recommend enrollment in a research protocol to identify additional mutations for patients in whom one or no mutations are identified. This abstract was funded in part by NIH/ORD CETT, MRG, RO1 HL071798 and U54 154 RR019480.

Age at diagnosis among individuals with hereditary and non-hereditary retinoblastoma. *S. Gilinsky, R. D. Clark, A. L. Murphree* Division of Ophthamology, Retinoblastoma Program, Children's Hospital Los Angeles, Los Angeles, CA.

Retinoblastoma (Rb), a malignant childhood tumor of the eye, affects approximately 1/15,000-1/20,000 live births in developed countries. This childhood eye cancer can affect one eye (unilateral) or both eyes (bilateral). All individuals with bilateral Rb and approximately 15% of individuals with unilateral Rb have a cancer-predisposing germline mutation in the *RB1* gene. The reported mean age of diagnosis of individuals with unilateral Rb is 24 months, and the mean age of diagnosis in bilateral Rb has been reported to be 12 months. We report the genetic status and age of diagnosis of 178 unilateral (n=143) and bilateral (n=35) Rb patients referred for genetic counseling. The average age of diagnosis among bilateral cases was 9 months, and among unilateral cases was 18 months. Seventeen of 102 sporadic unilateral Rb cases were positive for a germline mutation (16.7%). Of the 17 sporadic unilateral germline cases, 5 were mosaic, and 4 had cytogenetic deletions of 13q14. The median age of diagnosis among all unilateral germline mutation carriers (n=17) was 18 months. The median age of diagnosis among all unilateral non-carriers (n=85) was also 18 months. These data suggest that the *RB1* germline mutation status of the patient is not associated with the age at diagnosis in cases of unilateral Rb. This highlights the point that a child who presents with later onset unilateral Rb cannot be assumed to be a sporadic case. All individuals with sporadic unilateral Rb should be offered genetic testing regardless of age of onset. The discovery of a germline mutation in an individual with unilateral Rb could have implications for the patients risk for secondary cancers as well as for family members and future offspring of the patient. We hypothesize that bilateral retinoblastoma may be diagnosed earlier because of the higher likelihood of detecting an abnormality present in two eyes, rather than in unilateral Rb where only one eye is affected.

Molecular pathogenesis of Wolfram Syndrome different in different causal genes. *S. Amr, R. Shiang* Dept Human Genetics, Virginia Commonwealth Univ, Richmond, VA.

Wolfram Syndrome (WFS) is a debilitating autosomal recessive neurodegenerative disorder characterized by juvenile onset insulin dependent diabetes mellitus (DM) and optic atrophy (OA) as well as a number of neurological and endocrine complications that result in early death. Previous research has mapped Wolfram syndrome to chromosome 4p16.1 and the disease has been attributed to mutations the WFS1 gene affecting the WFS1 protein (wolframin), an ER membrane glycoprotein that plays an important role in intracellular Ca2+ homeostasis. Several studies of WFS1 mutant or knockdown cells revealed increased levels of the unfolded protein response (UPR) markers, increased apoptosis, and decreased proliferation. Furthermore, other research shows an upregulation of WFS1 upon activation of UPR elements. An additional locus for WFS on chromosome 4q22-24 was identified by linkage studies of 4 Jordanian Bedouin families with 16 affected individuals. We were able to identify the responsible gene in the critical region as CISD2, a gene encoding an ER intermembrane small protein (named ERIS) with a conserved iron-sulfur binding CDGSH domain. Using the pSUPER RNAi system (oligoengine) to knockdown CISD2, WFS in-vitro models were created in two cell lines; rat pancreatic insulinoma cells (INS1) and mouse neuroblastoma cells (N1E-115). Effective knockdown was measured by semi-quantitative RT-PCR and Real-Time PCR. Spliced XBP-1, a marker of the UPR, was not upregulated in CISD2 knockdown N1E-115 cells and CISD2 mutant lymphoblastoid cells taken from a WFS affected individual. Another marker, phosphorylated-eIF2, also did not show upregulation in the affected lymphoblastoid cells. CISD2 expression in wildtype cells treated with the ER stressor thapsigargin did not change compared with untreated controls. These data suggest that, unlike WFS1, CISD2 does not play a role in the UPR and mutant CISD2 results in WFS in a manner different than that in mutant WFS1. Possible alternatives to disease development, including changes in apoptosis and proliferation, are currently being studied in CISD2 knockdown cells.

CYP17, CYP1B1, CYP1A1 AND COMT POLYMORPHISMS AND THE SPONTANEOUS GENOMIC LESIONS IN BREAST CANCER WOMEN. *R. A. Santos[1], M. B. Mayorano[1], A. C. Teixeira[1], J. M. Andrade[1], H. H. A. Carrara[1], C. S. Takahashi[2]* 1) Faculty of Medicine, , Ribeirão Preto, São Paulo, Brazil; 2) Faculty of Philosophy Sciences and Letters of Ribeirão Preto, São Paulo, Brazil.

Breast cancer (BC) is the second most frequent form of cancer in worldwide and the most common malignant disease among women. Risk factors for BC include early age of menarche and late menopause, hormonal therapies, exposure to environmental pollutants, smoking and alcohol habits; however, increased or prolonged estrogen exposure is the most important risk factor. Estrogen biosynthesis and metabolism requires enzymatic pathways regulated by different genes with some polymorphisms described in association with BC. Estrogens increase the formation of DNA adducts, 8-oxo-dG and breaks on DNA molecule. Here we investigated the levels of DNA damage on untreated BC patients, the possible association of estrogen metabolizing genes CYP17, CYP1B1, CYP1A1 and COMT polymorphisms on BC risk and its influence on the spontaneous levels of DNA damage. DNA damage was detected by Micronucleus test and Comet Assay using peripheral blood lymphocytes from 45 diagnosed BC women and 85 healthy women. Micronucleus frequencies and DNA damage detected by Comet Assay were significantly higher in BC group than in controls. Polymorphism study was conduced in 131 healthy control and 104 BC women using PCR-RFLP. Polymorphisms in CYP17, CYP1B1, CYP1A1 and COMT were not different between patients and controls and CYP17 and CYP1A1 had no effect on basal DNA damage in both groups; the Leu allele in CYP1B1 was significantly associated with the higher levels of DNA damage in control group and did not affect DNA damage in BC group. Otherwise, in the control group individuals carrying the Met allele of COMT exhibited lower levels of DNA damage when compared to homozygous wild type, but in BC group the polymorphic homozygous individuals (Met/Met) presented higher levels of DNA lesions than their counterparts carrying the wild type allele. The present results suggest that BC women present an important genomic instability and estrogens metabolizing polymorphisms may modify the levels of DNA damage in healthy and in BC.

Influence of ethnicity on the OCA phenotype. *P.-W. Chiang[1], A.C-H Tsai[1], C. Clericuzio[2], E. B. Spector[1]* 1) Dept Pediatrics, UCD School of Medicine, Aurora, CO; 2) Pediatrics-Division of Dysmorphology, University of New Mexico School of Medicine.

The spectrum of pigmentation in humans is highly diverse. Pigmentation disorders, such as oculocutaneous albinism (OCA), are characterized by hypopigmentation of eyes, skin and hair and have been traditionally considered to be autosomal recessive disorders. The potential influence of genetic background (ethnicity) on the OCA phenotype and/or mechanism has not been addressed. More than 50% of Caucasian OCA1 and OCA2 patients have only one identifiable mutation. Almost every African American (Black) OCA patient has two identifiable mutations. A common allele in the Black population cannot explain the discrepancy. The Hispanic population, which carries a mixture of Caucasian, Native American and Black alleles, has rarely been studied. Our studies of 2 Hispanic families with OCA provide evidence that genetic background is essential in the determination of OCA phenotype and mutation mechanism. The first large Hispanic family studied carried the common Sub-Saharan African 2.7-kb deletion of OCA. The skin color of family members who carried the deletion (haplo-insufficient) was lighter compared with non-carrier family members, indicating that the effect of an OCA2 mutation on skin color is not strictly recessive. In a second Hispanic family two siblings were diagnosed as having OCA1B. They demonstrated variable expression of the OCA phenotype, even though they carried the same two OCA1 mutations. Molecularly, these two patients have autosomal recessive ocular albinism (AROA). Similar to Caucasian AROA patients, our patients carry one severe OCA1 mutation (G47D) and the hypomorphic R402Q allele. Unlike the reported Caucasian AROA patients, our patients are clearly hypopigmented. We hypothesize that the clinical spectrum of OCA depends upon pigmentation threshold. In individuals with darker complexion, two severe mutations are usually required to produce a visible phenotype. In individuals with lighter complexion, OCA can have a broader spectrum depending upon the presence of two mutations or the presence of one mutation plus a hypomorphic or ethnic specific allele.

Exploring genome-wide association and expression quantitative trait loci of cystatin C. *E. O. Kistner[1], D. L. Nicolae[2,3], A. Pluzhnikov[2], A. Tikhomirov[2], J. E. Below[4], A. Konkashbaev[2], C. Roe[2], M. E. Dolan[5], N. J. Cox[2,4]* 1) Dept Health Studies, Univ Chicago, Chicago, IL; 2) Section of Genetic Medicine, Dept. of Medicine, Univ Chicago, Chicago, IL; 3) Dept. of Statistics, Univ Chicago, Chicago, IL; 4) Dept. of Human Genetics, Univ Chicago, Chicago, IL; 5) Section of Hemotology/Oncology, Dept. of Medicine, Univ Chicago, Chicago, IL.

Serum cystatin C has been identified as a predictor of glomerular filtration rate in healthy individuals and those suffering from chronic kidney disease. Cystatin levels have also been shown to associate with cardiovascular endpoints, including hypertension and myocardial infarction. In order to identify risk variants in either chronic kidney disease or cardiovascular disease pathways, a genome-wide association study of serum cystatin C was conducted using the Genetics of Kidneys in Diabetes (GoKinD) cohort. This sample consisted of 1825 probands with type I diabetes, ascertained such that half the cohort suffered from diabetic nephropathy. The Affymetrix 5.0 SNP platform was used and tests of linear association between the inverse of serum cystatin C and additive genetic effects were conducted. The most significant association was found on chromosome 20 where the CST3, CST4, and CST9 genes are located (p=6.09E-07). Using the 90 human lymphoblastoid cell lines (LCLs) from the HapMap CEU population, expression quantitative trait loci (eQTLs) of the cystatin genes were explored. Single nucleotide polymorphisms (SNPs) predicting cystatin expression levels in LCLs were discovered using the publicly available SNP and CNV Annotations Network (SCAN) database serving Affymetrix Exon Array data. Cystatin eQTLs were found in regions on chromosome 2 that also show significant association with serum cystatin levels in type I diabetics (p<.0001). The protein kinase C (PRKCE) and Rap guanine nucleotide exchange factor (RAPGEF4) coding regions are implicated both as predictors of cystatin expression in LCLs and cystatin levels in the GoKinD cohort. The KEGG pathway database shows PRKCE inhibits the insulin signaling pathway. Further work is necessary to determine the mechanisms through which PRKCE and RAPGEF4 influence cystatin levels.

Congenital joint dislocations and chondrodysplasia caused by carbohydrate sulfotransferase 3 (CHST3) deficiency. *A. Superti-Furga[1], P. Hermanns[1], A. Megarbane[2], R. Mendoza-Londono[3], B. Afroze[4], A. Perez-Aytes[5], A. Rossi[6], L. Bonafé[7], L. Boccone[8], G. Nishimura[9], S. Hollander[1], J. Spranger[1], B. Zabel[1,10], S. Unger[1,10]* 1) Ctr Pediatrics, Univ of Freiburg, Freiburg, Germany; 2) Genet Medicale, Univ St. Joseph, Beyrouth, Lebanon; 3) Clin and Biochem Genetics, Hosp for Sick Children, Toronto, Canada; 4) Dept of Pediatrics, Aga Khan Univ Hospital, Karachi, Pakistan; 5) Hospital Infantil La Fe, Valencia, Spain; 6) Dept. of Biochemistry, Univ of Pavia, Pavia, Italy; 7) Molecular Pediatrics, Univ of Lausanne, Lausanne, Switzerland; 8) Clinical Genetics, Univ of Cagliari, Cagliari, Italy; 9) Dept. of Radiology, Tokyo Metropolitan Kiyose Children's Hosp, Tokyo; 10) Inst of Human Genetics, Univ of Freiburg, Freiburg, Germany.

We recently reported CHST3 gene mutations in children diagnosed with recessive Larsen syndrome and Humero-Spinal Dysostosis (Hermanns et al, AJHG 82:1368-74, 2008). We now report CHST3 mutations in four additional patients who had been diagnosed with Larsen syndrome (1 case), SED with luxations (1 case), and chondrodysplasia with luxations, "Megarbane type" (two cases; AJMG 130A:107-109, 2004, and AJMG 143A:1782-1787, 2007). The clinical features in our group of ten patients are homogeneous and include dislocation of the knees and/or hips at birth, clubfoot, elbow joint dysplasia with subluxation and limited extension, short stature, and kyphoscoliosis developing in late childhood. The recessive CHST3 mutations comprise missense, nonsense, and splicing mutations; pathogenicity of missense mutations was confirmed by the observation of impaired 6-O-sulfation of chondroitin sulfate proteoglycans in fibroblasts of three patients. CHST3 mutations had first been observed in an Omani kindred with a spondyloepiphyseal dysplasia variant (SED Omani type). Our results show that congenital luxations of knees, hips, and elbows are common and important diagnostic criteria for CHST3 deficiency, and that the condition is more common than hitherto believed and includes disorders variably diagnosed as recessive Larsen syndrome, Humero-Spinal Dysostosis, chondrodysplasia with luxations (Megarbane type), SED Omani type, and unspecified SED forms.

The Investigation of Risk Factors Associated with Nondisjunction of Chromosome 21 in Sperm and an Examination of the Excess of Males among Individuals with Down Syndrome. *T. Oliver[1], A. Bhise[1], E. Feingold[2], S. Tinker[1], N. Masse[1], S. Sherman[1]* 1) Dept Human Genetics, Emory Univ, Atlanta, GA; 2) Department of Human Genetics, University of Pittsburgh, Pittsburgh, PA.

Previous studies on relatively small samples of paternally-derived cases of live births with trisomy 21 have shown that: 1) advanced paternal age is not a risk factor for nondisjunction (NDJ) of chromosome 21, 2) absence of recombination, but not location of recombination, is associated with paternal NDJ and 3) an excess of males compared to females are born with paternally-derived trisomy 21. Some studies have suggested that the excess of males with paternally-derived trisomy 21 may explain the male excess observed among live births with trisomy 21 in general. We have studied 129 cases of trisomy 21 due to paternal nondisjunction to examine these three factors: paternal age, recombination and the male:female sex ratio. We genotyped STRs along 21q to identify the origin of the error and the location of recombination along the nondisjoined chromosome. We found among meiotically-derived paternal errors, 32% occurred in meiosis I (MI) and 68% in meiosis II (MII). We confirmed the lack of a paternal age association with either type of error (mean paternal age for MI errors, MII errors, and controls: 32.2 6.3, 30.6 6.5, 31.3 6.6, respectively). However, contrary to previous studies including our own, we did not find an association with recombination patterns among MI or MII cases of paternal NDJ. We found an increased male:female sex ratio among both paternal (1.28, 95%;CI: 0.68-1.91) and maternal (1.16, 95%;CI: 1.02-1.33) meiotic cases of trisomy 21. While the sex ratio in paternal cases is not statistically significant, taken together these findings suggest that the excess of males among live births with trisomy 21 may be due to selection against female fetuses with trisomy 21. The results from this study have enabled us to understand the risk factors associated with nondisjunction of chromosome 21 in sperm, in addition, they have provided insight on the origin of the excess of males observed among all cases of trisomy 21.

Distribution and prevalence of Lisch nodules in adults with NF1. *D. Stewart[1], S. Boley[2], J. Sloan[1]* 1) NHGRI/NIH, Bethesda, MD; 2) Vassar College, Poughkeepsie, NY.

The presence of 2 or more Lisch nodules (LN) is a diagnostic criterion for neurofibromatosis type 1 (NF1). No prospective, quantitative study has been performed on the distribution of LN in adults. Methods. Digital slit-lamp photographs of both eyes were obtained in 80 individuals enrolled in a natural history study of NF1. All patients met consensus criteria for the diagnosis of NF1, except 4 patients with missense mutations in NF1. LN were identified computationally and manually and LN characteristics (coordinates, area, diameter, distance to pupil center) were determined using ImageJ software. Eye color was determined by consensus from 3 observers. Results. Among post-pubertal individuals in our population, 93.6% had LN. Although the prevalence of LN increased with age, younger patients did not necessarily have fewer LN than older patients. Individuals with brown irides had a median of 2.0 LN while those with blue or green irides had a median of 16.3 LN. There were more LN in the inferior aspect than in the superior aspect of the iris ($p<0.0001$). There were more LN in the supranasal quadrant than the supratemporal quadrant ($p = 0.04$). In 4/7 sib pairs we observed a discordant number of LN. There were no significant differences in LN burden between the right and left eye. LN were not observed in any of the individuals with a missense mutation in NF1. Discussion. Our data supports the hypothesis that ultraviolet (UV) light exposure is needed for the development of Lisch nodules: 1) LN are most commonly located in the inferior portion of the iris, the region associated with the highest UV exposure, 2) the unequal distribution of LN across the superior portion of the iris (supranasal > supratemporal) is consistent with albedo (reflected sunlight) focusing by the cornea, 3) brown eyes have significantly fewer LN than blue eyes. In our dataset, 4.8% of adults lacked LN, a percentage observed in other studies. LN burden does not correlate with age. We hypothesize that haplo-insufficiency of NF1 and UV light are necessary but not sufficient for the development of LN. Variation in LN burden may be influenced by environmental factors and/or modifier genes.

Investigation on Mitochondrial tRNA genes of Iranian Multiple Sclerosis Patients. *S. EtemadAhari[1,2], M. Houshmand[1], M. Moin[3], M. Shafa Shariat Panahi[1], S. Kasraie[2]* 1) Dept Molecular Medical Genetic,National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran; 2) Sciences & Research Campus, Azad University, Tehran, Iran; 3) Immunology, Asthma & Allergy Research Institute, Tehran, Iran.

As with chromosomal DNA, the mitochondrial DNA (mtDNA) can contain mutations that are highly pathogenic .In fact, many diseases of the central nervous system are known to be caused by mutations in mtDNA. Dysfunction of the mitochondrial Respiratory Chain (RC) has been shown in patients with neurological disease including Alzheimers disease (AD), Parkinsons disease (PD) and Multiple sclerosis (MS). MS is a demyelinating disease of central nervous system characterized by morphological hallmarks of inflammation, demyelination and axonal loss. Considering this importance, we decided to investigate several highly mutative parts of mtDNA for point mutations as MT-LTI (tRNALeucine1(UUA/G)), MT-NDI (NADH Dehydrogenase subunit 1), MT-COII (Cytochrome c oxidase subunit II), MT-TK (tRNALysine), MT-ATP8 (ATP synthase subunit F0 8) and MT-ATP6 (ATP synthase subunit F0 6) in 20 IranianMSpatients and 80 age-matched control subjects by PCR and automated DNA sequencing to evaluate any probable point mutations. Our results revealed that 15 (75%) out of 20 MS patients had point mutations. Some of point mutations were newly found in this study. This study suggested that point mutation occurred inmtDNA might be involved in pathogenesis of MS.

Genetic and Immunologic Characterization of Gingiva in Systemic Hyalinosis. *T. C. Hart[1], R. Muralidharan[1], D. Pallos[2], S. I. Jang[1], S. K. Lee[1], P. S. Hart[3]* 1) NIDCR, NIH, Bethesda, MD; 2) University of Taubate, Sao Paulo, Brazil; 3) NHGRI, NIH, Bethesda, MD.

ANTXR2 mutations are etiologic for systemic hyalinosis (SH), clinically characterized by subcutaneous skin lesions, gingival hypertrophy, joint contractures and osteoporosis. Hyaline deposits of the dermis and other organs are pathognomonic. To understand gingival enlargement, which is seen in all SH cases, we identified ANTXR2 mutations in cases from 4 families. ANTXR2 isoform expression was determined with TaqMan assays. Immunohistochemical assays for 17 proteins associated with extracellular matrix, cell proliferation, apoptosis and vasculature development were performed in gingiva from SH cases and controls. Results: All SH cases were homozygous for a c.1074delT mutation, disrupting the cystolic domain. While ANTXR2 transcript levels were lower in SH, isoform expression profiles were similar in control (%) and SH [%] gingiva: ANTXR2-489 (84%),[90%]; ANTXR2-488 (15%), [10%]; ANTXR2-322 (<< 1%), [<<1%]. Immunolohistochemical assays revealed enlargement of SH gingiva occurred in the connective tissue, with decreased collagen and decreased fibroblast cell numbers ($p < 0.05$). Increased numbers of abnormal vascular vessels were seen, associated with amorphously hyalinized stromal tissue. Increased ANTXR2 expression was seen in hypertrophic SH gingival epithelium, connective tissue, and in the vasculature. Vasculature associated increased expression of extracellular matrix proteins, proliferative markers and apoptotic markers were noted. These findings suggest disruption of the ANTRX2 cystolic domain results in increased accumulation on the cell surface in SH tissue, particularly the vasculature. Immunohistochemical findings indicate abnormal proliferation and cellular apoptosis, resulting in defective vascular development in SH gingival enlargement. Finally, our findings indicate that SH associated gingival enlargement, characterized by decreased collagen, decreased fibroblasts and abnormal vasculature is distinct from gingival enlargement in more common forms of gingival fibromatosis, which demonstrate increased fibroblast numbers, increased collagen and normal vasculature.

Population genetics analysis of ALMS1 in humans reveals a surprisingly complex evolutionary history. *L. B. Scheinfeldt[1], S. Biswas[1], J. Madeoy[1], C. F. Connelly[1], E. E. Schadt[2], J. M. Akey[1]* 1) Dept Genome Sci, Univ Washington, Seattle, WA; 2) Rosetta Inpharmatics, LLC, a Wholly Owned Subsidiary of Merck and Co., Inc., Seattle, WA.

Mutations in the human gene ALMS1 result in Alström Syndrome, which presents with early childhood obesity and insulin resistance leading to Type 2 diabetes. Previous genome-wide scans for selection in the HapMap data suggest that ALMS1 was a target of recent positive selection. Through a detailed population genetics analysis of ALMS1 DNA sequence, haplotype, and SNP allele frequency variation in geographically diverse populations, we find that the signature of selection at ALMS1 is considerably more complex than what would be expected for an idealized model of a selective sweep acting on a newly arisen advantageous mutation. Specifically, we observed three highly divergent and globally dispersed ancient haplotypes (TMRCA ~ 1-3 mya), two of which carry a set of seven derived non-synonymous SNP alleles that are nearly fixed in Asian populations. Our data suggest that the interaction of human demographic history and positive selection on standing variation in Eurasian populations no later than 20 kya ago parsimoniously explains the spectrum of extant ALMS1 variation. Furthermore, our re-analysis of genome-wide SNP and gene expression datasets in humans and mice demonstrates that common inter-individual variation at ALMS1 is associated with metabolic phenotypes related to insulin resistance. These results provide new insights into the evolutionary history of ALMS1 in humans and suggest that selective events identified in genome-wide scans may be more complex than currently appreciated.

Statistical approaches for integrating gene-disease evidence into the medical care of individuals. *B. Mellen Snively*
Department of Biostatistical Sciences, Wake Forest University School of Medicine, Winston-Salem, NC.

In this era of ongoing genetic discoveries and widely available genetic tests, challenges exist for clinical translation to keep pace. For many genes and diseases, insufficient gene-disease information is available and ready for integration into clinical research and practice. The methods developed here aim to facilitate the appropriate use of genetic testing by 1) developing a flexible statistical framework that encompasses and enhances current tools for interpreting medical test outcomes in individuals, and 2) applying this framework to inform study designs to facilitate collection of gene-disease evidence applicable to interpreting genetic test outcomes. First, we developed and compared a set of competing estimators of the individual-specific likelihood ratio, LR=Pr(Observed test outcome|Phenotype present)/Pr(Observed test outcome|Phenotype absent); in diagnostic settings, LR is multiplied by a pre-test odds of disease to calculate the post-test odds and probability of disease. Then we simulated data to investigate study designs across allele frequencies, sample sizes, genetic models (general, additive, dominant, recessive) and LR thresholds. Applying the methods to TA-repeats of the *UGT1A1* promoter in colorectal cancer patients entering treatment with irinotecan (MIM 191740; N=250 in Toffoli et al., J Clin Oncol 2006), we observed similar LRs among estimators but not models. Under the general model, a novel profile LR for modifying the odds of an adverse drug reaction was 3.41, 1.33, and 0.421, and for a positive tumor response was 2.53, 0.938, and 0.889, respectively, given genotypes 7/7, 6/7, and 6/6 (generalizations to more complex tests are in progress). Differences among estimators were pronounced with smaller sample sizes, and size affected the probability of obtaining a LR of at least some threshold magnitude. In conclusion, our work provides study design tools specifically for the collection of gene-disease evidence applicable to interpreting genetic test outcomes in individuals. Furthermore, our work demonstrates the importance of close scrutiny of gene-disease measures with implications for genetic testing in clinical research and practice.

Expanding the Clinical Spectrum of Microgastria-Limb Reduction Complex. *S. Goobie[4], A. Koifman[1,2,4], S. Blaser[5], S. Keating[3], P. Shannon[3], S. Viero[3], L. Fishman[4], D. Chitayat[1,2,4]* 1) Prental Diagnosis and Medical Genetics Program, Mount Sinai Hospital, Toronto, ON, Canada; 2) Department of Obstetrics and Gynecology, Mount Sinai Hospital, Toronto, ON, Canada; 3) Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, Toronto, ON, Canada; 4) Division of Clinical and Metabolic Genetics, Hospital for Sick Children, Toronto, ON, Canada; 5) Department of Radiology, Hospital for Sick Children, Toronto, ON, Canada.

Microgastria-limb reduction defect is associated with (usually) upper limb defects including radial abnormalities, absent digits, generalized limb hypoplasia or amelia. Asplenia and splenogonadal fusion, as well as a variety of other abnormalities, have been reported. Microgastria can also be associated with VATER association. Although no specific etiology has been detected, it is obvious that this is an etiologically heterogeneous condition and in most cases sporadic. To the best of our knowledge, 15 cases with this condition have been reported in the English literature. Al-Gazali et al., (1999) reported two brothers from a consanguineous Sudanese family with features of the condition associated with hydrocephalus and agenesis of the corpus callosum, suggestive of an autosomal recessive mode of inheritance. We report 5 cases with this condition, and provide detailed clinical and autopsy findings which expand our knowledge about this rare condition. All of our cases presented with microgastria (5/5) and limb abnormality (ranging from lateral placement of the thumb to amelia). Renal abnormalities were uniformly present. All five cases had brain abnormalities and their karyotypes were normal. One case is a set of monozygotic twins who presented with the same abnormalities. Microarray analysis on the twin was normal thus excluding submicroscopic deletion or duplication detectable by the Signature Genomic chip. This supports the possibility that in some cases the condition is a single gene disorder with an autosomal recessive (or dominant) mode of inheritance.

Sixth female case of Myhre Syndrome. *M. Lopez-Cardona[1,4], A. Del Toro-Valero[1,2], G. Hernandez-Zaragoza[1], R. E'Vega-Hernandez[1,2], A. Feria-Velazco[3], J. C. Ledezma-Rodriguez[4], A. Bolaños-Muñoz[4], A. Macias-Anton[4], N. Davalos-Rodriguez[1,4]* 1) Instituto de Genetica Humana, Universidad de Guadalajara, Jalisco, Jalisco, Mexico; 2) Becario Conacyt; 3) Centro Universitario de Ciencias Biologías y Agropecuarias. Universidad de Guadalajara; 4) Hospital Regional Dr. Valentin Gomez Farias, ISSSTE, Jalisco, Mexico.

Lopez-Cardona MG1,4, Del Toro-Valero A1,2, Hernandez-Zaragoza1, EVega-Hernandez1,2, Feria-Velazco3, Ledezma-Rodriguez JC4, Bolaños-Muñoz A4, Macias-Anton A4, Davalos-Rodriguez N1,4. 1.- Instituto de Genética Humana. Universidad de Guadalajara 2.-Becario CONACYT 3.-Centro Universitario de Ciencias Biologías y Agropecuarias. Universidad de Guadalajara 4.- Hospital Dr. Valentín Gómez Farias, ISSSTE, Jalisco, México. Introduction: Myhre Syndrome (MS), a unknown rare heritance disease,characterized by short stature, mental retardation, low birth weight, facial dysmorphism, heart anomalies, early-onset mixed deafness, muscle hypertrophy and decreased joint mobility. Up to date 11 males cases and 5 females cases have been published. Objective: We describe the 6th female case and compare the clinical and radiological findings between all the patients. Case Report: The proposita a 26 year-old. In early infancy evidenced delay development and autoagression. At physical examination show short stature, microcephaly, mental retardation, maxillary hypoplasia, short palpebral fissures, short neck, bilateral thenar-hypothenar hypoplasia in hands, cutaneus syndactyly and brachydactyly and middle finger clinodactyly in the left hand, bilateral brachydactyly and syndactyly in 2nd and 3th toes. Radiological studies showed thick calvarium, broad ribs, hypoplastic iliac wings and hallux valgus. Discussion: The clinical and radiological characteristics present in the proposita suggested a diagnosis of MS, but she present new radiological characteristics. Even though, she isnt show deafness and microcephaly, and actually she wasnt muscular built.

Meta-Analysis of Genome-Wide Association Studies on Multiple Genotyping Platforms. *N. Zaitlen[1], B. Han[2], H. Kang[2], E. Eskin[3]* 1) Dept Bionformatics, Univ California, San Diego, La Jolla, CA; 2) Dept Computer Science, Univ California, San Diego, La Jolla, CA; 3) Dept Computer Science, Univ California, Los Angeles, Los Angeles, CA.

Genome-Wide association studies have successfully identified many novel loci associated with a variety complex human diseases. These associations have been of moderate to weak effect and therefore required the collection of large number of individuals. Recent analysis methods have leveraged the HapMap to improve the power of these studies by imputing genotypes of SNPs not contained on the genotyping platform. These imputation methods have also promised simple meta-analysis of association studies using different SNP sets, such as those on the Illumina and Affymetrix platforms. Meta-analysis would serve not only as means of validation and replication, but also improve the power of existing studies by vastly increasing the number of available individuals in a study. In this work, we show that straightforward meta-analysis of imputed genotypes causes significant inflation of p-values. We examine this problem in detail, determine which studies are most susceptible, and present two methods for addressing this problem. The first approach is permutation based requires complete access to the genotype data of both studies, and is computationally expensive. However, due to the sensitive nature of these data, we develop a second meta-analysis method which only requires the estimated genotype frequencies in the case and control populations. We show that the meta-analysis p-value inflation problem is due to heterogeneous errors of imputation across studies. We present a novel method for estimating the between study variance of imputed genotypes and use this variance to adjust the imputation based p-values during meta-analysis. We examine the severity of the problem and performance of our meta-analysis technique over simulation studies based on the Welcome Trust Case Control Consortium data. We show that our methods correct the distribution of p-values and improve the overall power of the studies.

Anthropological Insights from a Novel Visualization and Clustering Tool for HLA Haplotypes and Populations.

L. Gragert[1], V. Kumar[2], M. Steinbach[2], W. Klitz[3], M. Maiers[1], M. Ferdandez-Vina[4], S. Israel[5], C. Brautbar[5] 1) Bioinformatics, National Marrow Donor Program, Minneapolis, MN; 2) Computer Science, University of Minnesota, Minneapolis, MN; 3) Public Health, University of California, Berkeley, CA; 4) MD Anderson Cancer Center, Houston, TX; 5) Hadassah Medical Center, Jerusalem, Israel.

The HLA system has among the highest signal-to-noise ratios for population clustering in human genetics resulting from selection and demographic effects. We have applied methods inspired by gene expression array profile clustering to visualize and cluster HLA haplotype frequencies across human populations. The method first clusters haplotypes based on their frequency patterns in each population. The resulting haplotype clusters each contain a set of haplotypes that have a similar frequency pattern that is private to a subset of populations with the most recent common ancestry. A secondary clustering step independently creates a neighbor-joining tree of the populations to show their relatedness. The final matrix rearranges the haplotypes and populations to maximize block density and give clear human-interpretable signals. We applied this technique to Jewish populations from 32 countries and were able to make several important inferences about cryptic population history and migratory patterns. We also found that the haplotypes that clustered together were often recombinants or mutations of each other, so the common origin of many HLA haplotypes could be inferred. If datasets from multiple admixed and ancestral source populations are used, the composition of admixed populations and the source of their haplotypes can be determined. We conclude that this tool reveals information on both haplotype and population origins from large and complex datasets, giving the user rapid interpretation of HLA data for anthropological studies.

Phelan-McDermid syndrome in a patient with del(22)(q13.31q13.33) and an intact *SHANK3* gene. *F. M. Mikhail, R. D. Burnside, M. Descartes, A. J. Carroll* Department of Genetics, University of Alabama at Birmingham, Birmingham, AL.

Deletion 22q13.3 syndrome (Phelan-McDermid syndrome) is a microdeletion syndrome resulting from loss at 22q13.3 by simple deletion, unbalanced translocation, ring chromosome formation, or other unbalanced structural changes. The *SHANK3* gene maps to 22q13.33 and codes for a structural protein that plays a critical role in connecting ion channels and receptors in the post-synaptic membrane to the cytoskeleton and to signal transduction pathways. Haploinsufficiency of *SHANK3* has been proposed to be the cause of the major neurological features associated with deletion 22q13.3 syndrome. Here we report a 38-month-old boy with history of developmental delay, mild dysmorphic features and delayed expressive speech. The patient manifests many clinical features of deletion 22q13.3 syndrome including normal/accelerated growth, large prominent dysplastic ears, pointed chin, large and fleshy hands with thin deep set fingernails, hypoplastic toenails, and slightly decreased tone. HRB chromosome analysis was normal. Targeted array CGH analysis using the PerkinElmer constitutional chip v3.0 demonstrated an interstitial 22q13.3 deletion, which was confirmed by FISH analysis using the *ARSA* (22q13.33) and the 22q subtelomere probes. The *ARSA* probe showed a significantly diminished signal size both on metaphase chromosomes and in interphase nuclei indicating that the distal breakpoint mapped within the *ARSA* probe, whereas the 22q subtelomere probe showed a normal hybridization pattern. Using the 32k tiling path BAC array CGH chip, we were able to precisely map the breakpoints of our patients interstitial deletion, which was estimated to be ~5.2 Mb in size and to spare the most telomeric 205 kb on 22q including the *SHANK3* gene. In conclusion, our patient provides evidence that haploinsufficiency of *SHANK3* might not be the only candidate responsible for the majority of features in the deletion 22q13.3 syndrome, and that other critical genes proximal to *SHANK3* could have a major causal role in the phenotype. Detailed description of our patients clinical features, cytogenetic findings, and comparison with previously reported 22q13.3 deletion patients will be presented.

Dentatorubral-pallidoluysian atrophy in a family of African American ancestry. *K. A. Mraz[1], H. H. Wetzel[2], G. E. Hoganson[2]* 1) Northwestern University, Graduate Program in Genetic Counseling, Chicago, IL; 2) University of Illinois-Chicago, Department of Pediatrics, Division of Pediatric Genetics, Chicago IL.

Dentatorubral-pallidoluysian atrophy (DRPLA) is a rare neurodegenerative disease caused by a trinucleotide expansion in atrophin-1 on chromosome 12. Phenotypically, DRPLA is most similar to Huntington disease and is characterized by ataxia, choreoathetotic movements, and dementia. DRPLA occurs primarily in Japan and is considered extremely rare elsewhere. One exception is a large African American family from North Carolina in which DRPLA was genetically confirmed in the early 1990s. This family consists of five affected generations who have resided in the Haw River area of North Carolina since the mid-1800s and as a result, this variant of DRPLA has been classified as Haw River Syndrome. We report here what is predicted to be an additional family of African American ancestry recently identified as having DRPLA. This newly identified family, who primarily reside in the Midwestern United States, shows at least three affected generations, many with DNA confirmation of the atrophin-1 expansion. While the possibility of a distant relationship to the Haw River family remains, our family did not report any known ancestors in North Carolina and thus is presumed to represent an additional kindred with an expansion in the atrophin-1 gene and a phenotype consistent with DRPLA, or Haw River syndrome.

A two-stage, family-based candidate gene association study of left-sided heart defects. *K. McBride[1], G. Zender[1], S. Fitzgerald-Butt[1], S. Fernbach[2], A. Menesses-Dias[2], J. Towbin[2], J. Belmont[2]* 1) Dept Mol & Human Gen, Res Inst Nationwide Child, Columbus, OH; 2) Dept Mol & Human Gen, Baylor Coll of Med, Houston, TX.

Left-sided heart defects, including congenital aortic valve stenosis (AVS), coarctation of the aorta (COA), and hypoplastic left heart syndrome (HLHS), are common and carry significant morbidity and mortality. We have previously demonstrated a strong genetic component in these defects. A two stage association study was performed on a group of candidate genes from developmental pathways and processes important in cardiogenesis, to identify susceptibility genes for this group of defects. *First stage:* 199 genes were selected, based on animal models demonstrating a cardiac phenotype. The individuals consisted of 138 White and 58 Hispanic affected case-parent trios. 1977 SNPs were genotyped using the Molecular Inversion Probe methodology. TdT analysis with FDR correction for multiple testing in the White trios resulted in a total of 45 different genes with a $q<0.20$. ERBB3, B4, and NRG1 in the ERB pathway had positive association; ERBB3 & B4 were also associated in the Hispanic trios. Resequencing of the ERB3 & B4 and NRG1 genes did not identify specific disease-causing mutations. *Second stage:* A total of 139 tag SNPs were selected in the 3 ERB pathway genes. Genotyping was performed with ABIs SNPlex assay in the original cohort, plus a second cohort of trios (total White trios n=344; AVS n=102, COA n=134, and HLHS n=98; and total Hispanic n=101). FBAT analysis with correction by permutation demonstrated replication of the association for ERBB4. A 3 SNP high-risk haplotype in the 3 region of intron 3 was over-transmitted in both cohorts of White subjects (separately and analyzed together), and independently among those with AVS, COA and HLHS. Analysis of the Hispanic trios also demonstrated over-transmission of the haplotype, but did not reach statistical significance. This study confirms the positive association of ERBB4 in two cohorts with left sided heart defects as a group and individually by specific defect.

Genome-wide epistasis analysis of multiple sclerosis susceptibility. *W. Bush, J. Haines, M. Ritchie, IMSGC* Ctr Human Genetics Research, Vanderbilt Univ, Nashville, TN.

Evaluating epistasis in genome-wide association studies (GWAS) is an important yet difficult challenge in human genetics, as most common diseases have complex underlying genetic architectures that likely include small independent effects and interactions between many genes. The combinatorial explosion to test interactions among GWAS dataset SNPs prevents an exhaustive search. Thus, filtering the SNPs to identify a favored subset is a necessity. To address this problem we applied a bioinformatics approach for generating and ranking biologically supported multi-locus models of multiple sclerosis (MS) susceptibility. This approach automatically accesses and indexes data sources implying interaction of molecules, data sources implying gene relationships to disease, and literature-based data sources. These sources are integrated using genomic convergence and an implication index that measures the number of data sources that support the model. We constructed a set of putative gene-gene interactions based on the Kyoto Encyclopedia of Genes and Genomes (KEGG), Reactome, the Database of Interacting Proteins (DIP), the Protein Families Database (PFAM), the Gene Ontology (GO), and Netpath. We also constructed a set of putative disease-associated genes, using the Genetic Association Database (GAD), previous linkage screens of MS families, three studies of MS gene expression, and other candidates from literature-based sources. Using these sets, we identified 16.9 million multi-locus models for evaluation using conditional logistic regression in 931 MS case/pseudo-control pairs from the International Multiple Sclerosis Genetics Consortium. We demonstrate a highly significant relationship ($p < 0.001$) between the increasing number of data sources supporting a multi-locus model and model significance. This result strongly implicates genetic interactions as a significant source of MS susceptibility. Several individual models were highly significant and did not include known MS risk genes. Our approach can be applied to any GWAS dataset for rapid exploration of epistasis and will help further our dissection of the genetic architecture of complex disease.

Smith-Lemli-Opitz syndrome and autism: Correlation of neurocognitive measures with plasma and CSF sterols.

F. D. Porter[1], S. E. Sparks[2], A. Aneja[3], H. Goodwin[1], L. E. Kratz[3], W. E. Kaufmann[3], E. Tierney[3] 1) NICHD, NIH, DHHS, Bethesda, MD; 2) CNMC, Washington, DC; 3) KKI, Baltimore, MD.

Smith-Lemli-Opitz syndrome (SLOS) is a recessive malformation syndrome with a specific behavioral phenotype that includes autistic findings in most patients. SLOS is an inborn error of cholesterol synthesis where mutations of *DHCR7* impair the reduction of 7-dehydrocholesterol (7DHC) to cholesterol. Thus SLOS patients have both a deficiency of cholesterol and an accumulation of 7DHC. The neurocognitive defects in SLOS are likely due to a combination of fixed neurodevelopmental defects and functional deficits due to the abnormal sterol biochemistry in the brain. Because therapeutic interventions can improve the sterol biochemistry, it is critical to establish the relationship between the neurocognitive defects and sterol levels. We hypothesized that the autistim features, adaptive behavior deficits, and IQ would correlate with sterol levels. To test this, we measured plasma and CSF sterol levels in 15 SLOS subjects for whom we had concurrent results of the ADOS, ADI-R, Mullen or Stanford-Binet and Vineland. Linear regression analyses of variables: CSF cholesterol/total sterol (C/TS) ratio: ADOS ($p<.04$); ADI-R present score (ADI)($p<.0001$); FSIQ ($p<.001$); Vineland Adaptive Behavior Composite (VABC) $p<.002$). CSF cholesterol: ADOS (Not significant (NS)); ADI ($p<.04$); FSIQ ($p<.03$); VABC (NS). Plasma Cholesterol: ADOS (NS); ADI (NS); FSIQ ($p<.001$); VABC ($p<.0004$) Plasma C/TS ratio: ADOS ($p<.04$); ADI ($p<.0004$); IQ ($p<.0001$; VABC ($p<.0003$). Subsequent anlaysis showed significant correlations between CSF 7DHC, but not cholesterol, and the social and non-verbal communication subdomains of the ADI. This data show that in SLOS the sterol abnormality correlated with increased autistic features, decreased IQ, and increased adaptive behavior deficits. Thus, the neurocognitive deficits in SLOS may be a functional consequence of the abnormal biochemistry in the brain, and potentially amendable to therapeutic interventions that improve the biochemical defect. These observations may have broader implications in that low cholesterol levels have been reported in non-syndromic autism.

High resolution reconstruction of HLA haplotypes in Native Americans. *W. Klitz[1], L. Gragert[2], M. Maiers[2], B. Tu[3], J. Ng[3], C. Hurley[3]* 1) Public Health, University of California, Berkeley, CA; 2) National Marrow Donor Program, Minneapolis, MN; 3) Georgetown University, Washington, DC.

It is not uncommon for a defined ethnic group to be the product of admixture between two or more parental populations occurring one or many generations in the past. Here we examine Mexican Americans--residents of the United States who have immigrated from Mexicoand reconstruct the HLA haplotype frequencies of their Native American ancestors. Mexican Americans are composed of a combination of ancestral Native American, European and African source populations, at estimated proportions of 44%, 48% and 8% respectively. Sequence based typing on HLA A, C, B and DRB1 was performed on each of 553 self-described Mexican Americans. A-B-DRB1 haplotypes frequencies were estimated. Existing European American and African American A-B-DRB1 high resolution haplotype frequency data was used to remove the Europe and African source haplotypes from the Mexican American data, leaving haplotype frequencies representing Native American founder A-B-DRB1 haplotypes HLA alleles and haplotypes previously characterized as native American were evident. Curiously, of the most frequent 40 A-B-DRB1 haplotypes, ten were present one or more times in the large sample of Europeans, many of these at similar or even higher frequencies. Closer examination of these cases showed that these instances are all likely present in the modern Spanish population at frequencies higher than in the more northerly origin European sample used. The high resolution data represent the best and probably most knowable sample of Native American HLA from the time of the European invasion 500 years ago.

***SHANK3* haploinsufficiency may be responsible for as much as 10% of cases of pervasive developmental disorder not otherwise specified (PDD-NOS).** *L. Boccuto[1,2], M. Lauri[2], C. D. Skinner[1], D. Buccella[2], J. S. Collins[1], M. Zollino[2], R. J. Schroer[1], G. Neri[2], R. E. Stevenson[1], F. Gurrieri[2], C. E. Schwartz[1,3]* 1) Greenwood Genetic Center, Greenwood, SC; 2) Istituto di Genetica medica, Universitá; Cattolica del Sacro Cuore, Rome, Itlay; 3) Department of Genetic and Biochemistry, Clemson University, Clemson, SC, USA.

Autism Spectrum Disorders (ASD) include three main conditions: autistic disorder, pervasive developmental disorder not otherwise specified (PDD-NOS), and Asperger syndrome. In recent years, many genes associated with ASD have been identified. Notably, some of these genes are involved in the neuroligin-neurexin interaction at the glutamate synapse: *NLGN3*, *NLGN4*, *NRXN1*, and *SHANK3*. We screened two cohorts of ASD patients - 131 patients from the South Carolina (SC) and 74 from Italy - for mutations in the *SHANK3* gene, a component of post-synaptic density. We found seven cases with *de novo* alterations: a c.1349C>T (p.P450L) transition in one SC patient with autistic disorder and two Italian patients with PDD-NOS, a c.3895delG (p.Glu1299fs) mutation in one Italian patient with PDD-NOS, three large intragenic deletions in three Italian patients with PDD-NOS. The overall mutation rate was 3.4%, three-fold higher than previously reported in autism patients. There were some significant differences in mutation rates by clinical phenotype. The mutation rate was 0.7% in cases with autistic disorder and 10.3% in cases with PDD-NOS. Thus, *SHANK3* mutations appear to be responsible for a significant number of PDD-NOS cases, with a lesser prevalence in patients with autistic disorder. We also noted some features - overgrowth at birth, tendency to obesity, and absent or delayed speech - shared by the patients carrying *SHANK3* mutations, are compatible with the diagnosis of 22q13 deletion syndrome, confirming a potential role of this gene in the neurological symptoms of 22q13 deletion syndrome. *SHANK3* is the first gene that exhibits evidence of a genotype/phenotype correlation in ASD. This suggests that a detailed clinical-neuropsychiatric evaluation may be an important tool for effective genetic screening of ASD patients.

Clinical Diagnostic *PORCN* Gene Sequencing for Focal Dermal Hypoplasia. *P. H. Fernandes[1,2], V. R. Sutton[1,2], P. A. Ward[1,2], C. M. Eng[1,2], I. B. Van den Veyver[2,3], P. Fang[1,2]* 1) Medical Genetics Laboratories; 2) Department of Molecular and Human Genetics; 3) Department of Obstetrics and Gynecology, Baylor College of Medicine, Houston, TX.

Focal dermal hypoplasia (FDH) or Goltz syndrome is an X-linked dominant disorder characterized by defective development of ecto-mesodermally-derived tissues. The gene responsible for FDH was recently identified as *PORCN*, which encodes an enzyme that enables membrane targeting and secretion of Wnt proteins. Approximately 80% of FDH-causing mutations in *PORCN* are single nucleotide substitutions, small deletions or small insertions, identifiable by sequence analysis of the *PORCN* coding region. Up to 20% of FDH patients have large genomic deletions that include *PORCN*. We implemented clinical diagnostic sequencing for *PORCN* mutation analysis in September 2007 and thus far have studied samples of 27 females and 5 males that were referred for a clinical diagnosis of FDH. Mutations that were either previously reported or predicted to be disease-associated were found in 15/32 samples (47%). These included 7 nonsense mutations, 5 previously reported missense mutations, 1 small deletion, 1 small insertion, and 1 splice site mutation. Two of these mutations were found in affected males and were mosaic, 13 were presumed heterozygous in females. Mutations were *de novo* in all 3 cases where parental studies were performed. One previously reported mutation, p.G60R, was identified 3 times in apparently unrelated families and appears to be recurrent. We also identified novel unclassified variants in 4 patients. One was a *de novo* missense p.H341Y change in a female patient, not seen in either parent. We also found a c.555+4A>G change that is predicted to disrupt the exon 5 splice donor site in two affected half-siblings. No mutation was identified by sequence analysis in 13 probands (40%). Further studies to evaluate large gene deletions are indicated in these patients. These results indicate that *PORCN* gene sequencing for a clinical diagnosis of FDH detects point mutations in up to 60% of cases.

ENZYME REPLACEMENT THERAPY FROM BIRTH IN MUCOPOLYSACCHARIDOSIS IVA MICE: EARLY TREATMENT LEADS TO REMISSION OF BONE LESIONS. *S. Tomatsu, A. Montaño, H. Oikawa, A. Ohashi, V. Chi Dung* Dept Pediatrics, St Louis Univ, St Louis, MO.

Mucopolysaccharidosis IVA (MPS IVA) is a lysosomal storage disorder characterized by severe systemic bone disease, resulting in short stature, bone deformities, and pulmonary compromise. Early treatment is vital to prevent long-term clinical pathology in lysosomal storage disorders. We used MPS IVA mice to assess the effects of long-term enzyme replacement therapy initiated at birth. Two recombinant human N-acetylgalactosamine-6-sulfate sulfatase (GALNS) produced in CHO cell lines were used: native GALNS and sulfatase-modifier-factor 1 (SUMF1) modified GALNS. MPS IVA mice received weekly intravenous injections of 250 units/g body weight of recombinant human GALNS until 14 wk of age. After doses of 250 units/g body weight were administered intravenously, each enzyme was primarily recovered in liver and spleen, with detectable activity in other tissues including bone and brain. MPS IVA mice treated from birth by 14 weekly intravenous injections of SUMF1-GALNS demonstrated almost no lysosomal storage in most tissues including heart valves, sinus lining cells in bone marrow, osteoblasts, osteocytes, and connective tissues surrounding the articles and ligaments. We obesrved reduction of the storage materials in chondrocytes. MPS IVA mice treated from birth kept the normal level of serum keratan sulfate. These data suggest that the enzyme which enters the cartilage before the cartilage cell layer becomes mature is able to protect against accumulation of storage material in MPS IVA mice. In conclusion, early treatment at birth leads to remission of bone pathology in MPS IVA mouse.

Reliable detection and detailed characterization by the Affymetrix SNP Array 6.0 of a wide variety of cytogenetic abnormalities in samples from the NIGMS Human Genetic Cell Repository. *J. Leonard[1], J. Veitch[2], N. Gerry[1], P. Bender[1], D. Coppock[1], N. Faravashi[2], M. Christman[1], J. Collins[2]* 1) Coriell Cell Repositories, Coriell Inst Medical Research, Camden, NJ; 2) AFFYMETRIX, INC., Santa Clara, CA.

The Affymetrix SNP Array 6.0 was used to define the chromosomal segments gained and lost in cultures in the Chromosomally Abnormal Collection of the NIGMS Repository. Cultures with known deletions or duplications, detected by other molecular analyses, and cultures from subjects with apparently balanced chromosomal rearrangments associated with abnormal phenotypes were also analyzed. Data were analyzed using both Affymetrix Genotyping TM Console Software and Partek Genomics Suite. To date, 380 unbalanced chromosomal abnormalities detected by G-band cytogenetic analysis were detected by the microarray. The band assignments of molecular breakpoints were compared with the cytogenetic calls using the UCSC Genome Browser. There was a high degree of consistency between the breakpoints involved in the abnormalities as called by the microarray and by the G-band analyses. In approximately 90% of the cases the breakpoints coincided or were in adjacent sub-bands. In addition thirty sub-microscopic deletion or duplication abnormalities previously determined by FISH or PCR were detected. In each case the segments detected were of the expected size and contained genes in the critical regions for the respective disorders: Williams Beuren, Wilms Tumor, Angelman, Prader-Willi,Charcot Marie Tooth A1, Miller-Dieker, Smith-Magenis, DiGeorge, Velocardiofacial, X-linked Ichthyosis, Duchenne Muscular Dystrophy, Pelizaeus-Merzbacher, and Azospermia. Samples from an additional forty-five subjects with phenotypic abnormalities and apparently balanced chromosomal abnormalities were also studied. Megabase sized deletions were found in the vicinity of the putative breakpoints in eight cases and five deletions were found at other sites. Evaluations of smaller changes are in progress.

The female Gaucher patient: recommendations for management of reproductive events. *P. Kaplan[1], A. Zimran[2], E. Morris[3], E. Mengel[4], N. Belmatoug[5], D. A. Hughes[6], V. Malinova[7], R. Heitner[8], E. Sobreira[9], M. Mrsić[10], S. Granovosky-Grisaru[2], S. vom Dahl[11]* 1) Ped/Metabolic, Childrens Hosp, Univ Pennsylvania, Philadelphia, PA; 2) Jerusalem, Israel; 3) Cambridge, UK; 4) Mainz, Germany; 5) Paris, France; 6) London, UK; 7) Prague, Czech Republic; 8) Johannesburg, South Africa; 9) Genzyme São Paulo, Brazil; 10) Zagreb, Croatia; 11) Cologne, Germany.

Background: Manifestations of type 1 Gaucher disease [GD] (bleeding, anemia, spleno-hepatomegaly & bone disease) may be relevant for female reproductive events. Aim: To advise on optimal management of females with GD based on data from women treated with alglucerase &/or imiglucerase (enzyme replacement [ERT]) or not treated. Methods: Clinicians experienced in GD reviewed peer-reviewed literature, a Canadian survey, an alglucerase-imiglucerase pharmacovigilance database & their own clinical data. Results: Menarche may be delayed in untreated girls with GD (average 14yrs untreated; 13.6yrs with ERT compared with 12-13yrs in general population). Menorrhagia is common & may worsen anemia. ERT can ameliorate menorrhagia alone (2 p=0.043) or with oral contraceptives (2 p=0.001). There is no evidence of decreased fertility. Literature on 356 untreated and 42 treated pregnancies and 338 untreated & 78 treated pregnancies in our patients show women can have successful pregnancies but in untreated GD symptoms may worsen and increased spontaneous abortions, pre & post partum hemorrhage, infection, and complications from splenomegaly may occur. Data show reduced risk with ERT of spontaneous abortion (2 p = 0.008), delivery complications (2 p <0.0005) & GD-related post partum complications (2 p = 0.014) compared with untreated. There is no evidence of adverse effects of ERT on the fetus or breast fed infants. Menopause data are lacking. Conclusions: ERT reduces menorrhagia & pregnancy complications in GD. Symptomatic patients needing ERT should achieve therapeutic goals before conception & have multidisciplinary care of pregnancy. ERT treated women may be advised to continue ERT in pregnancy & breast feeding. GD bone disease in menopause requires further study.

A Pyrosequencing assay to rapidly and quantitatively assess methylation at KvDMR1. *D. K. Bourque[1], L. Avila[1], M. S. Peñaherrera[1], D. E. McFadden[2], P. von Dadelszen[3], W. P. Robinson[1]* 1) Medical Genetics; 2) Pathology and Laboratory Medicine; 3) Obstetrics and Gynaecology, University of British Columbia, Canada.

KvDMR1 is the centromeric imprinting control region (ICR2) on chromosome 11p15.5 and is involved in the regulation of several genes, including *CDKN1C*. Methylation of this region is usually measured at a differentially methylated *NotI* restriction enzyme cutting site using Southern blotting. This differentially methylated region has previously been reported to be hypomethylated in some cases of Beckwith-Wiedemann syndrome (BWS). We have developed a Pyrosequencing assay to rapidly and quantitatively measure methylation at seven CpG sites in KvDMR1, including the BWS diagnostic *NotI* site. The assay is highly reproducible ($r=0.98$, $p<0.0001$) and was validated in several BWS cases, two of which showed reduced methylation (27% and 10%) as compared to blood controls (70%, N=11). Eight cases of Silver-Russell syndrome (a syndrome of growth deficiency) showed normal methylation. We also assessed KvDMR1 methylation in placentas from pregnancies affected with either fetal intrauterine growth restriction (IUGR) or preeclampsia (PET) because genes on 11p15.5 are known to be involved in fetal and placental growth and development. However, we found no significant difference in mean methylation level between the placental groups: control (N=22), 65.4%; IUGR (N=13), 64.8%; PET (N=17), 65.2%; PET+IUGR (N=21), 64.7%. Trophoblast and mesenchymal cells from one complete hydatidform mole (CHM) of androgenetic origin were also assessed with reduced methylation levels of 8% and 30%, respectively. This suggests that while the paternal allele is generally unmethylated, there may be some paternal methylation present in the mesenchymal cells, at least in this case. Placental samples from three cases of androgenetic chimerism (identified as placental mesenchymal dysplasia) also showed reduced methylation only in the samples identified as having significant levels of androgenetic cells. The rapidity and reproducibility of this Pyrosequencing assay should make it a useful tool in the timely diagnosis of KvDMR1 methylation errors in BWS and CHMs.

Pentraxin 3 genetic variation is associated with dizygotic twinning in The Gambia: linking innate immunity with super-fertility in a pleiotropic model. *G. Sirugo[1,2,3], D. R. Velez[4], K. K. Ryckman[4], C. Bisseye[1], H. Chapman[1], A. Worwui[1], M. Diatta[1], G. Morris[1], R. Agedbola[1], K. Odunsi[5], G. Page[6], S. M. Williams[4]* 1) Dept Human Genetics, Medical Res Council Labs, Banjul, Gambia, Gambia; 2) Medical Genetics Unit, Ospedale S. Pietro FBF, Rome, Italy; 3) Tor Vergata University, Rome, Italy; 4) Vanderbilt University, Nashville, TN; 5) Roswell Park Cancer Institute, Buffalo, NY; 6) University of Alabama, Birmingham, AL.

Dizygotic (DZ) twinning has a known genetic component and its frequency is particularly high in West Africans, up to 4% of live births in Nigerian Yoruba. However, no genes have so far been identified that would explain the peculiar frequency of the trait in sub-Saharan Africa. In The Gambia the frequency of DZ twinning is about 2% of live births, without epigenetic effects due to the use of fertility drugs or to consumption of food known to induce ovulation. Pentraxin 3 (PTX3), a soluble pattern recognition receptor which plays a functionally important role in both innate immunity and female fertility, is a candidate gene for DZ twinning in a tropical setting where prevalence of infectious diseases and twinning is high. In order to investigate the role of PTX3 in Gambian women with DZ twins we typed a set of five PTX3 intragenic SNPs in 113 sister pairs who had DZ twins (98 full sister and 15 half-sister pairs). Significant linkage between PTX3 and DZ twinning was found with markers rs1840680 and rs3845978 by ASP analysis (P=0.01 and P=0.0003 respectively). We also compared inferred haplotype frequencies in subjects with DZ twins to frequencies from 95, ethnically diverse Gambians: subjects with DZ twins were found to significantly differ from controls (P=0.003 for the 5 SNPs-extended haplotype spanning PTX3). Our results suggest that PTX3 variation affects DZ twinning in Gambians: selective pressure on PTX3 variants modulating immune responses to infectious agents could explain the high frequency of DZ twinning in West Africa.

Recurrent 15q13 microdeletion CNV: a susceptibility locus for autism, mental retardation, and psychiatric abnormalities. *S. Ben-Shachar[1], B. Lanpher[2], JR. German[1], M. Shinawi[1], CW. Cheung[1], JR. Lupski[1], AL. Beaudet[1], T. Sahoo[1]* 1) Molecular & Human Genetics, Baylor College of Medicine, Houston, TX; 2) Department of Pediatrics, Vanderbilt University, Nashville, TN.

Deletions of chromosome 15q11.2q13 result in Angelman or Prader-Willi syndromes (AS and PWS). Recurrent deletions within 15q13, immediately distal to the AS/PWS critical region, are associated with a novel microdeletion syndrome whose clinical features include mental retardation, seizures, facial dysmorphisms, and mild digital abnormalities. The consistent size (~1.6 Mb) and clustering of breakpoints at low copy repeats (LCRs) suggest that the rearrangements are mediated by non-allelic homologous recombination (NAHR). We identified 14 individuals with microdeletions of 15q13 (11 children including a pair of siblings and 3 parents) by testing of over 12,000 clinical samples by array-CGH. The children had neurological and psychiatric phenotypes including mental retardation (10/11), autistic spectrum disorder (5/11), speech delay, aggressiveness, and other behavioral problems. The deletion was *de novo* in one family, inherited from an apparently normal mother in two families, and inherited from a father with cognitive impairment and bipolar disorder in a fourth family. Familial origin is presumed in two affected siblings with the same deletion and phenotype; thus the deletion was inherited in four of five families implying high heritability and possible incomplete penetrance. Interestingly, 7 of the 11 affected probands were not living with their biological parents, and most of the 11 biological parents unavailable for testing had a history of social, cognitive and psychiatric disabilities including bipolar disorder, depression, antisocial behavior, and schizophrenia. We hypothesize that additional some of these parents, including one parent of the affected sibs, harbor the deletion. These results reveal a major novel locus not only for a syndromic form of autism, but also a strong association with neuropsychiatric problems. Further analysis of the involved genomic interval and the genes contained therein may provide a better understanding of their role in these phenotypes.

Rapid and accurate genomewide p-value correction and power estimation with correlated markers. *B. Han[1], H. Kang[1], E. Eskin[2]* 1) Dept Computer Sci, Univ California, San Diego, La Jolla, CA; 2) Dept Computer Sci and Human Genetics, Univ California, Los Angeles, CA.

With the development of high-throughput genotyping technologies, current genetic association studies typically involve genotyping hundreds of thousands of markers. The large number of correlated markers bring to the forefront two variants of the multiple-hypothesis testing correction problem, p-value correction and per-marker threshold estimation. Permutation test, the gold standard, is computationally impractical for a large dataset. Several recent studies efficiently solve the problem by utilizing the multivariate normal distribution to simulate the null distribution of test statistic. These approaches straightforwardly scale to the whole genome by partitioning the region into blocks. However, by assuming independent blocks, the correlations between blocks are ignored which may lead to inaccuracies. Moreover, it is not possible to estimate the null distribution of p-values with previous methods, which requires repeated application of the method to each p-value. In this article we propose a novel method for multiple-hypothesis testing correction and power estimation called SLIDE which takes into account all correlations within a sliding window. Since SLIDE estimates the null distribution of p-values, every p-value of interest can be simultaneously corrected. Our simulations show that SLIDE is two orders of magnitude faster than the previous methods, and reduces the errors in the corrected p-values by 38% on average. We show that power estimation can also be solved by the multivariate normal distribution framework. SLIDE estimates genomewide power with the same accuracy as standard empirical simulations of power, and is faster up to two orders of magnitude. Connecting the two different problems, multiple-hypothesis testing correction and power estimation, provides useful insights that the per-marker threshold estimated from a reference dataset can approximate the true per-marker threshold. Our method is publicly available at **http://slide.cs.ucla.edu**.

Patterns of Genetic Variation at *TAS2R38*, a Bitter-Taste Receptor Gene, in Diverse African Populations. *M. Campbell[1], A. Ranciaro[1], A. Froment[2], D. Drayna[3], P. Breslin[4], S. Tishkoff[1,5]* 1) Department of Genetics, University of Pennsylvania, Philadelphia, PA; 2) Muséum National d'Histoire Naturelle-Centre National de la Recherche Scientifique, Paris, France; 3) National Institute on Deafness and Other Communication Disorders, National Institutes of Health, Rockville, MD; 4) Monell Chemical Senses Center, Philadelphia, PA; 5) Department of Biology, University of Pennsylvania, Philadelphia, PA.

An important dietary adaptation in human populations is the ability to taste bitter compounds. A hypothesized selective advantage of bitter taste perception is that it helps individuals to avoid ingesting toxic substances in plants. Although studies have examined *TAS2R38*, a taste receptor gene associated with perception of the bitter substance PTC, in a number of geographic populations, little is known about patterns of variation at this locus in diverse populations from sub-Saharan Africa. In this study, we sequenced a 3.0 kb region encompassing the *TAS2R38* receptor locus in a number of culturally and linguistically diverse populations from Cameroon and Kenya. We also examined correlations between genotypic variation and bitter taste phenotypes (perception of PTC) in these populations. Our study shows that African populations have a higher degree of nucleotide and amino acid haplotype variability compared to previous studies of non-African populations. The presence of several amino acid haplotypes at high frequency across diverse African populations is also consistent with balancing selection. Furthermore, our sliding window analysis of Tajimas D values suggests that variants at the 5' and 3' ends of the coding region may be the targets of selection. Additionally, the diverse haplotypes present in Africans are correlated with a broader range of taste sensitivity than is typically observed outside of Africa, enabling us to assess the effect of particular amino acids on taste perception. Overall, this study provides further information regarding the genetic basis of taste perception in Africa and the role that genetic and phenotypic variability may play in adaptation to diverse diets.

Molecular and functional analysis of a novel MEK2 mutation in cardio-facio-cutaneous syndrome: Transmission through four generations. *K. A. Rauen[1], A. L. Estep[1], S. J. Bale[2], W. E. Tidyman[1], Y. Lacassie[3]* 1) Department of Pediatrics, UC San Francisco, CA; 2) GeneDx, Gaithersburg, MD; 3) Department of Pediatrics, LSUHSC and Childrens Hospital New Orleans, LA.

Cardio-facio-cutaneous (CFC) syndrome is a rare MCA disorder in which individuals have characteristic dysmorphic features, cardiac defects, ectodermal anomalies and delay. CFC is caused by alteration of activity through the Ras/mitogen-activated protein kinase (MAPK) pathway due to heterozygous de novo mutations in protein kinases B-Raf, MEK1 or MEK2; the majority occur in BRAF, whereas mutations in MEK1 or MEK2 comprise about 27%. We report a 7 mo male with a clinical diagnosis of CFC. Bidirectional sequence analysis of MEK2 revealed a novel c.383C>A transition in exon 3 resulting in a nonsynonymous missense substitution p.P128Q. Upon further evaluation, other family members including the probands mother and half-sib displayed phenotypic features of CFC. Genomic DNA from 11 members of this four generation family were screened for the MEK2 mutation identified in the proband. Nine family members with characteristics of CFC including the mother and half-sib tested positive for the MEK2 mutation; 2 family members who did not have features of CFC including the probands father and a maternal great aunt did not. SIFT analysis determined MEK2 p.P128Q to be deleterious. To corroborate the functional alteration of the novel mutant protein, transient transfection of 293T cells with subsequent Western analysis demonstrated increased kinase activity as measured by ERK phosphorylation. The p.P128Q mutant had increased pERK compared to wildtype. The level of pERK was less than the constitutively active MEK2 S222D/S2226D mutant and CFC MEK2 p.F57C mutant which is known to have a high level of activity indicating MEK2 p.P128Q is weakly hypermorphic. This is the first identified vertically transmitted functional CFC MEK mutation reported further expanding our understanding of germline mutations within the Ras/MAPK pathway. Our findings underscore the importance of a thorough genetic evaluation of family members and that activating mutant proteins within the MAPK cascade may be compatible with human reproduction.

-308 TNF promoter polymorphism predicts outcome in patients with rheumatoid arthritis treated with the anti-TNF agent etanercept but not infliximab. *A. G. Wilson[1], C. Potter[2], K. L. Hyrich[2], A. Barton[2], J. Worthington[2], J. D. Isaacs[3], A. W. Morgan[4], J. R. Maxwell[1], BRAGGSS* 1) University of Sheffield, Sheffield, United Kingdom; 2) University of Manchester, Manchester, United Kingdom; 3) Newcastle University, Newcastle, United Kingdom; 4) University of Leeds, Leeds, United Kingdom.

Background: The introduction of agents designed to inhibit tumour necrosis factor (TNF) has revolutionised the treatment of patients with severe Rheumatoid Arthritis (RA). These therapies are however expensive, and 30% of patients fail to respond. There has therefore been great interest in identifying markers predictive of treatment efficacy, which would allow targeting of these treatments to those most likely to benefit. We have therefore investigated whether genotypes of single nucleotide polymorphisms (SNPs) in the region containing the TNF gene predict response to anti-TNF therapy in RA. Methods: In a large cohort of Caucasian RA patients treated with anti-TNF medications (n=1,050) eight candidate SNPs were genotyped. Linear regression analyses adjusted for baseline 28 joint disease activity score (DAS28), baseline health assessment questionnaire (HAQ) score, gender and concurrent disease modifying anti-rheumatic drug (DMARD) treatment, were used to assess association of these polymorphisms with response to anti-TNF therapy, defined by change in DAS28 after 6 months of treatment. Analyses were performed in the entire cohort, and also stratified by anti-TNF agent. Results: Association between DAS28 response and TNF -308 genotype (p=0.001) was detected across the whole cohort. After stratification by anti-TNF agent, the TNF -308AA genotype was associated with a significantly poorer response compared with TNF-308GG in Etanercept (p=0.001) but not Infliximab (p=0.8) treated patients. No significant associations were detected for the other SNPs. Interpretation: We conclude that TNF -308 genotype is associated with efficacy of Etanercept but not Infliximab in the treatment of RA. These data may facilitate therapeutic targeting of these expensive medications.

Candidate gene association studies aimed at identifying genetic modifiers of penetrance in DYT1 primary dystonia. *S. Gavarini[1], A. Clark[1], D. Raymond[2], A. Mitchell[1], S. B. Bressman[2,3], L. J. Ozelius[1]* 1) Department of Genetics and Genomic Sciences, Mount Sinai School of Medicine, New York, NY; 2) Department of Neurology, Beth Israel Medical Center, New York, NY; 3) Department of Neurology, Albert Einstein College of Medicine, Bronx, NY.

The most severe form of the inherited primary dystonia is early onset, generalized torsion dystonia. A three-base pair deletion (946delGAG) in the gene encoding torsinA (*TOR1A*) is responsible for most cases of the disease, but only 30-40% of mutation carriers exhibit dystonic symptoms. To date, age of onset is the single reliable factor related to the prognosis of primary dystonia. Despite the identification of the *DYT1* gene, pathological changes that lead to symptoms remain unknown. D216H, a coding-sequence variation at the *TOR1A* locus, has been shown to contribute to the incomplete penetrance in mutant-gene carriers. However, its overall contribution to explaining reduced penetrance is modest suggesting other factors contribute to DYT1 dystonia. *TOR1A* is highly homologous to three other genes, *TOR1B*, *TOR2A* and *TOR3A*. These homologous genes may be able to compensate for some torsinA functions in non-manifesting carriers. Genes coding for protein interactors of torsinA are also strong candidates for mediating the genetic influence on penetrance. Here we report the results of an association study comparing non-manifesting versus manifesting carriers of the *TOR1A* mutation looking at single nucleotide polymorphisms (SNPs) within torsin-related genes and genes encoding known protein interactors of torsinA. Identification of genes influencing the penetrance of DYT1 dystonia should clarify the underlying mechanisms of the disease and provide prognostic markers that would be clinically valuable in relation to potential future pharmacological treatments.

Genetic polymorphisms in the Diasporin genes and breast cancer survival. *J. R. Long[1], X. O. Shu[1], Y. Gao[2], C. Li[3], S. Qu[1], Z. X. Ruan[2], Q. Cai[1], W. Zheng[1]* 1) General Internal Medicine, Vanderbilt Univ, Nashville, TN; 2) Department of Epidemiology, Shanghai Cancer Institute, Shanghai, China 200032; 3) of Biostatistics, Vanderbilt University, Nashville, TN 37232.

Recent data from gene expression analyses have suggested that the Diasporin pathway play an important role in tumor progression. Candidate genes involved in this pathway include the NDN, PI16, LUC7L, RRP1B, BRD4, CENTD3, SIPA1 and CSF1R. Utilizing data from an ongoing genome-wide association study of breast cancer, we investigated the association of genetic polymorphisms of these genes with breast cancer survival in a cohort of 754 patients recruited from 1996 to 1998 and were followed for a median of 7.1 years as part of the Shanghai Breast Cancer Study (SBCS). A total of 63 SNPs located in these 8 genes plus flanking 10kb and having a minor allele frequency 0.05 are included in the Affymetrix SNP 6.0 array. Five SNPs were found to be associated with breast cancer disease-free survival (DFS) at a significance level of 0.05. All of these 5 SNPs are located in the CSF1R gene, including rs1010102, rs13360152, rs6579772, rs11743220, and rs17798680. The protein encoded by CSF1R gene is the receptor for colony stimulating factor 1, a cytokine which controls the production, differentiation, and function of macrophages. There is strong LD between rs6579772 and rs11743220, all the other pairwise r2 are less than 0.5. After adjustment for age and clinic predicators, i.e. TNM stage, ER, PR status and therapy, improved DFS was seen for patients with the rs13360152 minor allele; patients with heterozygote (HR: 0.8, 95% CI: 0.6-1.1) and homozygote (HR: 0.6, 95% CI: 0.3-0.9) fared better than patients with common allele homozygote (Ptrend=0.03). Patients carrying the minor allele of the other 4 SNPs had a worse prognosis than those with major allele. SNP rs1010102 showed the strongest effect, with HR (95% CI) being 1.3 (0.9-1.9) for heterozygote and 4.0 (1.9-8.6) for homozygote (Ptrend=0.004) with comparison to homozygote for the common allele. These results are promising and are being validated in an independent sample of patients.

Mutations in *FAM83H* in a cohort of autosomal dominant hypocalcified amelogenesis imperfecta families. *P. S. Hart[1], S. Cinar[2], D. Cogulu[2], D. Ozdemir-Ozenen[3], E. Firatli[4], T. Han[5], P. Sulima[5], D. L. Domingo[5], T. C. Hart[5]* 1) NHGRI, NIH, Bethesda, MD; 2) Ege University, Izmir, Turkey; 3) Yeditepe University, Istanbul, Turkey; 4) University Istanbul, Istanbul, Turkey; 5) NIDCR, NIH, Bethesda, MD.

Amelogenesis imperfecta (AI) is a heterogeneous group of disorders that affect the amount or quality of dental enamel. A total of 14 subtypes have been recognized historically based upon the clinical phenotype and mode of inheritance. Recently mutations in *FAM83H* were described in 3 Korean, 2 Caucasian and 1 Hispanic kindred segregating autosomal dominant hypocalcified AI (ADHCAI). We studied 1 North American and 7 Turkish kindreds with the same phenotype and identified 2 novel mutations, p.Q444X (1 Turkish family) and p.Q456X (6 Turkish families), as well as a previously described p.W460X mutation (North American family). In the 6 families with the p.Q444X mutation, haplotype analysis was undertaken to determine if this mutation represented a founder mutation or a mutational hotspot. In 5 of the 6 families, it was determined that the mutation had been inherited from a common ancestor, although the families could be split into 2 distinct groups based upon the amount of haplotype sharing. In the sixth family, the proband was the only affected member of his family, suggesting that the mutation arose as a *de novo* event, an assumption supported by the haplotype data which revealed a distinct haplotype. These results bring to 8 the number of mutations described in *FAM83H*. Only 2 mutations, p.Q456X and p.W460X, have been found in more than one kindred. The p.Q456X mutation was determined to be a founder mutation in Turkey and as an apparent *de novo* mutation in another Turkish family. A *de novo* mutation was also reported by Lee et al. 2008. The finding of 2 *de novo* mutations out of a total of 8 mutations suggests that there may be a high mutation rate in this gene. Consistent with the findings of Kim et al 2008 and Lee et al 2008, the mutations found in the Turkish families were nonsense mutations. This raises the question of whether the underlying molecular mechanism is haploinsufficiency or a dominant negative effect.

Complex rearrangements in Pelizaeus-Merzbacher disease suggest a coupled homologous and nonhomologous repair mechanism. *G. Hobson[1], K. McLean[1], L. Banser[1], D. Lavoie[1], J. Garbern[2], K. Sperle[1]* 1) Nemours Biomedical Research, A I duPont Hosp Children, Wilmington, DE; 2) Wayne State University, Detroit, MI.

The X-linked leukodystrophy Pelizaeus-Merzbacher disease (PMD) is most frequently caused by duplication of the proteolipid protein 1 gene (*PLP1*). Our data and that of others suggest that these duplications are a result of a coupled homologous and nonhomologous repair mechanism (CHNR) involving a double-strand DNA break and error-prone nonhomologous end-joining (NHEJ), as microhomologies are frequently found at the junctions between duplicated regions. More recently it was suggested that many rearrangements in the *PLP1* region are complex and that they are refractory to junction analysis. A replication-based mechanism termed Fork Stalling and Template Switching (FoSTeS) involving a single-strand DNA break was proposed for these rearrangements. We present our analyses of junction regions in PMD patients with complex rearrangements by semiquantitative PCR, array CGH and interphase FISH. The complex rearrangements include interrupted duplicated regions, triplications, and higher copy numbers. We argue that the CHNR mechanism is involved in the formation of complex rearrangements.

Possible role of TOMM40L in Alzheimers disease risk: Investigation of multiple SNPs in TOMM40L/APOA2 region. *L. M. Bekris[1], N. M. Galloway[2], C. E. Yu[1,2]* 1) Dept Med, Univ Washington, Seattle, WA; 2) Veterans Affairs Puget Sound Health Care System, Seattle, WA.

Multiple SNPs in the TOMM40/APOE gene region are in linkage disequilibrium and are the source of the strongest genetic association with late-onset Alzheimers disease (LOAD) risk. Recently, we have reported a possible mechanistic link between TOMM40 SNPs and LOAD risk whereby APOE protein levels have been associated with SNPs within the TOMM40 gene suggesting the existence of an APOE regulatory element within the TOMM40 gene. Alternatively, there could be an independent effect of the Tom40 protein, encoded by the TOMM40 gene, on LOAD risk. A TOMM40 homolog (TOMM40L) has recently been identified that appears to be a functional component of the Tom40 mitochondrial membrane complex. The TOMM40L gene is located next to APOA2 near a linkage signal for LOAD. Thus, in this investigation we hypothesize that because genetic loci within the TOMM40/APOE gene region are associated with LOAD, genetic loci within the TOMM40L/APOA2 gene region may also be associated with LOAD. Specifically, the aim of this study was to genotype SNPs (n=5) within the TOMM40L/APOA2 gene region and test for association between these SNPs and LOAD patients that have at least two family members with LOAD (FAD; n=474), sporadic LOAD patients without a family history of LOAD (SAD; n=272) and cognitively normal controls (n=878). Three SNPs were found to be marginally associated with SAD risk; rs3813627 (OR: 1.29, 95% CI; 0.99-1.70,); rs3813628 (OR: 1.26, 95% CI; 0.96-1.66) and rs4233368 (OR: 0.80, 95% CI; 0.61-1.05) but not FAD risk. While taking into account gender and APOE 4, rs6413453 and rs3813627 predict SAD risk but not FAD risk. FAD females with the rs3813628 C allele have an earlier FAD age-at-onset that is independent of APOE 4. In summary, an association with both LOAD risk and LOAD age-at-onset was found with SNPs located within the TOMM40L region. An association between TOMM40L SNPs and LOAD suggests that either, TOMM40L and thus mitochondrial function has a direct impact on LOAD risk, or the paralog regions of TOMM40/APOE and TOMM40L/APOA2 share some molecular mechanism that influences LOAD risk.

HLA Haplotype Diversity in Brazil. *M. Maiers[1], L. Gragert[1], W. Klitz[9], M. Elisa Moraes[3], M. Gerbase-DeLima[4], C. Vergueiro[5], M. daGraca Bicalho[6], S. Jamil Haddad do Monte[7], M. Torres[8], M. Ferdandez-Vina[2]* 1) Bioinformatics, National Marrow Donor Program, Minneapolis, MN; 2) MD Anderson Cancer Center, Houston, TX; 3) LIG, Immunogenetic Laboratory, São Paulo, Brazil; 4) Federal University, São Paulo, Brazil; 5) FCM, Santa Casa, São Paulo, Brazil; 6) Federal University of Paraná, Curitiba, Brazil; 7) Federal University of Piaui, Teresina, Brazil; 8) Albert Einstein Hospitla, São Paulo, Brazil; 9) Public Health, University of California, Berkeley, CA.

We have analyzed HLA allele and haplotype frequencies of a cohort of 116 895 Brazilian individuals in 4 racial/ethnic categories: African (3766), Asian (1376), European (87 321), Mixed Race (13 251) (Cafuso, Mulato, Pardo/Mestizo) who reside in 3 stratified geographical regions: North (8832), South (32 901), Southeast (75 162). Haplotype frequency analysis was performed using the EM algorithm on DNA based typing results for HLA-A, -B and -DRB1. Fst was computed on combined A, B, DRB1 2-digit allele frequencies. We applied a 2-D clustering method that analyzes both genetic variation and population variation simultaneously. The clustering output revealed several general patterns. Regional variation between North and South/Southeast was more distinguishing than race for defining clusters with the exception of Asian origin which formed a unique cluster. Non-European Northern populations defined three distinct clusters. The Southern and Southeastern groups appeared to have higher proporti The significant differences between Brazilian populations within the country and in comparison with US populations stress the need to develop and maintain a local stem cell registry to allow Brazilian patients access to well HLA matched donors.

Identifying genetic determinants of diabetic complications through genome-wide association studies of the Genetics of Kidneys in Diabetes (GoKinD) collection. *M. Kalscheur[1], J. Below[1], A. Konkashbaev[1], A. Pluzhnikov[1], C. Roe[1], A. Tikhomirov[1], E. Kistner[2], D. Nicolae[1], N. Cox[1]* 1) Department of Medicine, University of Chicago, Chicago, IL; 2) Department of Health Studies, University of Chicago, Chicago, IL.

Introduction: Genetic predisposition likely plays a role in the development of diabetic complications. Identifying loci that contribute to this predisposition through a genome-wide association study (GWAS) may provide greater understanding of the pathogenesis, prevention and treatment of these complications. **Methods:** The GoKinD genetics collection contains information from close to 1900 individuals with long-standing (10+ years) type 1 diabetes (T1D) and a variety of complications including nephropathy, cardiovascular disease and peripheral vascular disease. Each study participant was genotyped using the Affymetrix Genome-Wide Human SNP Array 5.0. Quality control studies were performed to ensure sample quality, identify highly related samples, and explore population stratification. Allelic association tests that incorporated logistic regressions were performed to identify loci of interest. Phenotypes reported here include cardiovascular disease that resulted in intervention (CVDI; 91 cases, 636 controls) and peripheral vascular disease (PVD; 314 cases, 1322 controls). SNPs were evaluated based upon level of significance, quality control parameters, and robustness across covariates. **Results:** No SNPs were associated with either phenotype at a level reaching genome-wide significance. For CVDI, three SNPs reached p values $< 10^{-5}$. rs8032656 (15q26, p value = 6.2×10^{-6} to 2.6×10^{-5}) was of high quality and is located in an intron of *SLCO3A1*, a gene that encodes a transporter of prostaglandins, along with four other SNPs with p values < 0.0001. Three of these SNPs exhibit a high degree of linkage disequilibrium (LD) with both a missense and a frameshift mutation within *SLCO3A1*. For PVD, five SNPs produced p values $< 10^{-5}$. **Conclusion:** This GWAS of two T1D complications failed to identify any SNPs associated with disease at genome-wide significance; however, several interesting SNPs were found that merit further evaluation.

Natural selection on the erythrocyte surface glycoproteins in malaria-endemic human populations of Africa. *W.-Y. Ko, K. Kaercher, S. A. Tishkoff* Department of Genetics, School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA.

Understanding the evolutionary mechanisms underlying host-parasite competition is fundamental for studying the origin and spread of infectious disease. Detecting signatures of natural selection at genes involved in host-parasite interaction may help identify variants that play a critical role in the invasion pathways. In humans, glycophorin A and B are the major glycoproteins expressed on the surface of erythrocytes and are thought to be receptors that can be recognized by several pathogens, including the *Plasmodium falciparum* parasite causing malaria. Here, we resequenced three homologous glycoproteins - glycophorin A (*gypa*), B (*gypb*), and E (*gype*) in 285 individuals across 15 African populations. Because of possible copy-number variation, which may bias our sequence analyses, we conducted quantitative PCR experiments and identified 4 individuals that may carry more than one copy in at least one of the three loci examined. Sequence analysis for the remaining individuals shows extraordinarily high genetic variation, where the estimates of average pairwise nucleotide differences () are 0.0030, 0.0024, and 0.0017 for *gypa*, *gypb*, and *gype*, respectively. Sliding-window analysis of Tajimas *D* statistic reveals an excess of intermediate-frequency SNPs at exon 2 of *gypa* (that codes for the extracellular domain) in several malaria hyper-endemic populations but not in hypo-endemic populations. Interestingly, two replacement SNPs identified at this region are located at the positions that determine the MN blood type. Our results provide strong evidence that balancing selection is maintaining MN blood-type alleles in malaria endemic populations. In addition, despite >90% sequence similarity among these homologs, we observe evidence for a different type of selection (positive directional selection) acting on the same homologous region of *gypb*, where an excess of rare-frequency SNPs was found in 3 populations. Sequence comparison among the three genes shows that gene conversion may have played a role in maintaining genetic diversity upon which natural selection could act.

Genetic and Environmental Influence on Serum Lipid Tracking: A Population-based, Longitudinal Twin Study.

S. Zhang[1,2], X. Liu[1,2], J. Necheles[1,2], H. J. Tsai[1,2], G. Wang[1,2], B. Wang[1,2], Z. Li[3], X. Liu[3], X. Wang[1,2] 1) Smith Child Health Program, Children's Memorial Hosp, Chicago, IL; 2) Department of Pediatrics, Northwestern University Feinberg School of Medicine, Chicago, IL; 3) Institute for Biomedicine, Anhui Medical University, Hefei, China.

Background: Serum lipid tracking during childhood and from childhood to adulthood has been examined. However, limited studies have explored genetic and environmental influence on serum lipid tracking from childhood to adolescence. Objective: This study examined the degree of serum lipid tracking from childhood to adolescence and genetic and environmental influence on the tracking. Methods: This study included a total of 520 same-gender twin pairs who were 6 to 12 years at the baseline, and who completed both baseline and follow-up study (with ~ 6 year interval). Assessment included anthropometric, zygosity, Tanner staging and serum lipid (LDL, HDL, cholesterol, and TG) measures. Generalized estimating equations were used to examine the lipid tracking from childhood to adolescence. A Bivariate Cholesky decompositions model was applied to estimate the genetic and environmental contributions to the lipid variation at each time point and phenotypic tracking correlation. Results: Participants with high tertile lipid levels at baseline tended to remain high at follow-up across all ages and Tanner stages, while subjects with low tertile at the baseline tended to remain low at follow-up. Both genetic and environmental factors influenced the phenotypic variation for the lipids. In addition, genetic component contributed to most of the phenotypic tracking for TC, TG and LDL except for HDL, whereas common environmental factors also play a role. Conclusions: Serum lipid profile showed significant tracking from childhood to adolescence. Such phenotypic tracking was largely influenced by genetic factors. This study underscores the importance to consider both genetic and environmental factors in order to identify early precursors of dyslipidemia and to design effective clinical and public health interventions.

Use of a Rett syndrome checklist in patients referred for clinical MECP2 testing. *D. Waggoner, M. Dempsey, A. Platteter, S. Das* Human Genetics, Univ Chicago, Chicago, IL.

Rett syndrome (RTT) is one of the most common neurodevelopmental disorders in females. The clinical picture is complicated by atypical variants of RTT which have widened the phenotypic spectrum. Mutations and deletions of the X-linked *MECP2* gene are found in approximately 90% of patients with RTT. The diagnosis is not always readily apparent and Huppke et al. (2003) developed a 10-item checklist of RTT features with a score ranging from 0-12. Results of the checklist comparing the clinical features between patients with *MECP2* mutations and those without indicated that if genetic testing had been done on only those individuals with a score of 8 or higher on the checklist, 100% of mutation carriers would have been detected, and only 54% of non-carriers would have been tested. The patients used to develop the checklist included those with classic symptoms of RTT. Given the expanding phenotypic spectrum of RTT some patients with mutations may not have the classic features of RTT. We studied the utility of the Huppke checklist in 68 patients (21 mutation positive and 47 mutation negative) referred to The University of Chicago molecular diagnostics laboratory for clinical *MECP2* mutation analysis. The results showed that 14 of 21 (66%) mutation-positive patients had a clinical checklist score of 8 or greater or in other words 7 patients would have been missed by using the 8 point cutoff. Only two of the 10 necessary criteria had statistically significant differences between the two groups, namely stereotypic hand movements and normal psychomotor development in the first 6 months. Four of the seven mutation-positive patients with a checklist score less than 8 had one or both of these findings. The published RTT checklist may be a useful tool for diagnosing a child with classic RTT, but may miss patients that have atypical RTT. This study suggests modifications in the criteria for evaluating whether *MECP2* mutation analysis is warranted. In addition to using the suggested cutoff score of 8 points or greater, we suggest that the features of normal psychomotor development and stereotypic hand movements be assessed independently.

Structural Equations As a General Framework for Modeling Phenotype and Genotype Networks. *X. Fang[1], L. Luo[2], J. Reveille[3], M. Xiong[2]* 1) Mathematical Sciences, Peking University, Beijing, China; 2) Human Genetics Center, University of Texas School of Public Health, Houston, TX 77030; 3) Division of Rheumatology, Medical School, University of Texas Health Science Center at Houston, Houston, TX 77030.

Diseases are a process of environmental exposure and its interaction with genetic factors. Modern molecular epidemiology measures large number of biological parameters indicating a series of events along the causal chain between exposure and disease. These measures are referred to as biomarkers or intermediate phenotypes. To incorporate intermediate phenotypes into genetic studies of complex traits will facilitate identification of disease genes. In traditional genetic analysis, one or several phenotypes are used as dependent variables, and multivariate statistical methods are used to identify genes influencing phenotypes. However, such analysis cannot simultaneously model phenotypes as both dependent and independent variables and hence cannot fully employ phenotype information. In this report, we propose to use structural equations as a general framework for modeling phenotype and genotype networks to decipher the path from genomic information to the final outcome of the disease. The model consists of two steps. At the first step, we assume that the structural relations among phenotype and genotypes are known. Then, we use two stage least square estimate method to estimate the parameters in the equations. At the second stage, we use genetic algorithms to search the structures of phenotype and genotype networks. By iterations between two stages, we finally identify phenotype and genotype networks. By hypothesis testing we can identify the genes that influence the phenotype variations. The proposed methods are applied to ankylosing spondylitis (AS) data set. We found that IL23R was associated with the phenotypes of BASDAI, BASFI, PAINSCAL and COMORB index that influence the severity and progress of AS.

Gene Screening at the 13q32 Keratoconus Locus. *B. A. Bejjani[1], L. B. Wallis[1], K. A. Bailey[1], K. Cywinska[1], A. Molinari[2], J. A. Pitarque[2], B. Yue[3], M. Gajecka[1,4]* 1) WWAMI, Washington State Univ, Spokane, WA; 2) Hospital Metropolitano, Quito, Ecuador; 3) University of Illinois at Chicago College of Medicine, Chicago, IL; 4) Institute of Human Genetics, PAS, Poznan, Poland.

Keratoconus (KTCN) is a non-inflammatory thinning and anterior protrusion of the cornea that results in steepening and distortion of the cornea, altered refractive powers, and altered visual acuity. We ascertained eighteen autosomal dominant multigenerational KTCN families from Ecuador and identified a novel locus on 13q32.1-q32.3. We narrowed the critical region to a 5.6 Mb interval. Here we present sequencing results of candidate keratoconus genes localized to 13q32. The KTCN locus contains 23 known transcripts. Twelve of the genes were chosen for the evaluation based on known functions and putative functional implications to the pathophysiology of KTCN: *FARP1, MBNL2, FGF14, ZIC5, ZIC2, EFNB2, RNF113B, DOCK9, PHGDHL1, VGCNL1, ERCG5*, and *ING1*. Genes were screened by standard techniques using genomic DNA samples from twenty three individuals from the family that exhibited the highest LOD score at this locus and selected affected and unaffected individuals from other Ecuadorian keratoconus families. Coding exons and intron-exon boundaries of the genes were examined. To date, sequencing analysis of genes has not revealed mutations segregated with the disease phenotype. Several novel single nucleotide polymorphisms were identified.

Liver differences at gestation day 19 in a mouse model of an inborn error of metabolism, GKD, suggests a moonlighting function for the GK protein. *N. MacLennan[1], A. Presson[1,2], S. Horvath[2,3], E. R. B. McCabe[1,3,4]* 1) Pediatrics; 2) Biostatistics; 3) Genetics; 4) Bioengineering; and California NanoSystems Institute, UCLA, LA, CA.

Glycerol kinase deficiency (GKD) results in increased glycerol. The dogma is that maternal enzyme should protect the fetus in utero by removing glycerol. Work from us and others suggests the GK protein has moonlighting functions beyond its enzyme activity. We proposed to identify liver networks disrupted by Gyk KO prior to birth to determine if network alterations are seen prenatally, which would suggest involvement of moonlighting functions in GKD pathogenesis. We performed microarray expression analysis on embryonic day (e) 19 KO mouse livers and compared gene expression levels to those of day of life (dol) 3 livers. Gene filtering (dChip) identified 324 differentially expressed genes (1.35 fold change) at e19. Ingenuity Pathways Analysis (IPA) revealed differential gene expression in novel network pathways at e19. The top enrichment category for e19 liver expression was apoptosis (pe-6). Pathways significantly altered at e19 between WT and KO were apoptosis, lipid and amino acid metabolism, and cell signaling (pe-32 to e-43). Gyk was in the hepatic system disease pathway (pe-15) at e19. Glucocorticoid receptor signaling was the fifth highest canonical pathway at e19 (p0.01). IPA confirmed prenatal alterations of several known postnatal network pathways. Intersection of e19 and dol3 KO networks revealed a common apoptosis network with Gyk modeled as an integral part. Plasma glycerol levels were significantly different between KO and WT at both e19 and dol3, but e19 KO glycerol did not exceed dol3 levels. In conclusion, we show enrichment of apoptosis genes and glucocorticoid receptor signaling, proposed moonlighting functions of GK in prenatal life in Gyk KO mice. Plasma glycerol levels at e19 and dol3 suggest maternal enzyme is protecting the fetus by removing glycerol, but not protecting the fetus from loss of Gyks moonlighting functions. These alterations may be responsible for onset of GKD pathogenesis by in utero disruption of these moonlighting functions.

Identification of a novel BRAF fusion gene in pediatric low-grade astrocytomas. *A. J. Sievert[1], A. C. Resnick[2], T. H. Shaikh[1], J. A. Biegel[1]* 1) Children's Hospital of Philadelphia, Philadelphia, PA; 2) University of Pennsylvania School of Medicine, Philadelphia, PA.

Pediatric low-grade astrocytomas (LGA) are a heterogeneous group of tumors whose pathogenesis is not well understood. Distinguishing benign and malignant gliomas can be difficult, and there has been a lack of molecular markers to aid in diagnosis. We recently described a non-random ~2Mb duplication in 7q34 in 20 of 28 juvenile pilocytic astrocytomas and diffuse fibrillary astrocytomas using the Illumina 550K SNP-based oligonucleotide array platform. Validation of the duplication by FISH demonstrated a variety of patterns in the tumors, most consistent with a tandem duplication. Based on the SNP-array results, the proximal breakpoints were clustered in a region that contains KIAA1549, an uncharacterized gene. Distal breakpoints clustered within the BRAF locus. The BRAF oncogene is involved in regulation of the mitogen activating protein kinase (MAPK) pathway. Two common activating mutations have been described in a variety of solid tumors. One LGA had a V600E BRAF mutation with no 7q34 duplication. As both KIAA1549 and BRAF are transcribed in the same orientation, we hypothesized that a tandem duplication from KIAA1549 to the BRAF gene locus could result in a novel fusion gene. Reverse transcriptase PCR and sequencing was performed on tumors with and without the 7q34 duplication. Sequence analysis of a 1kb fusion product revealed a fusion between exon 15 of KIAA1549 and exon 11 of BRAF. Interestingly, the fusion product was identified in one sample that was normal by SNP-array analysis but demonstrated 3 copies of BRAF by FISH. The predicted KIAA1549-BRAF fusion gene includes the BRAF kinase domain but lacks the auto inhibitory N-terminal portion. This could result in constitutive activation of BRAF and subsequent enhanced activation of the MAPK pathway. In summary, we have identified a novel KIAA1549-BRAF fusion gene in a series of LGAs with a 7q34 duplication. Further studies are required to characterize the function of this fusion protein and determine the diagnostic and prognostic significance in the development of pediatric low-grade astrocytomas.

An audit of PTPN11 mutations in Noonan Syndrome and LEOPARD syndrome in the UK. *T. Khan, J. Short, R. Poh, R. Taylor, K. Kalidas, A. Crosby, M. A. Patton* Medical Genetics, St George's Hospital , London , United Kingdom.

The Regional Genetics Service at St George's Hospital in London has provided a national molecular genetic service for testing for Noonan syndrome with PTPN11 mutation testing and recently testing for SOS1, KRAS, and RAF1. The laboratory has tested for PTPN11 mutations in 343 individuals referred with a possible clinical diagnosis of Noonan syndrome. PTPN11 mutations have been identified in 30%. The mutations were in exons 3,4,7,8,12 and 13 but the majority of mutations occur in exons 3 and 8. Pulmonary stenosis occurred in 71% and hypertrophic cardiomyopathy in 7%. Five patients were referred with Juvenile Myelomonocytic Leukaemia (JMML). In these cases mutations in exon 3 were found in 3 patients (214G>C, 214G>T and 182A>G), one had a mutation in exon 4 (417G>C) and one had a mutation in exon 8 (854T>C). In addition there were 31 individuals referred with a possible clinical diagnosis of LEOPARD syndrome and PTPN11 mutations were identified in 74%. In most cases the mutations were in exons 7 and 12 but in five cases the mutation was in exon 13. One patient with a mutation in exon 12 (1403C>T) illustrates the difficulty of classification as she had multiple lentigines, pulmonary stenosis with facial features of Noonan syndrome and also clinical and histological evidence of neurofibromatosis. The audit indicates the mutations are non-random and there is an emerging pattern of genotype-phenotype correlation.

Identification of the gene for the cblF defect of vitamin B12 metabolism reveals a novel lysosomal membrane protein with homology to lipocalin receptors. *F. Rutsch[1], S. Gailus[1], I. Racine-Miousse[2], T. Suormala[3], C. Sagné[4], M. Toliat[5], G. Nürnberg[5], T. Wittkampf[1], I. Buers[6], A. Sharifi[4], M. Stucki[7], C. Becker[5], M. Baumgartner[7], H. Robenek[6], T. Marquardt[1], W. Höhne[8], B. Gasnier[4], DS. Rosenblatt[2], B. Fowler[3], P. Nürnberg[5]* 1) Dept Pediatrics, Univ Children's Hosp, Münster, Germany; 2) Dept Human Genetics, McGill Univ, Montreal, Canada; 3) Dept Biochem, Univ Children's Hosp, Basel, Switzerland; 4) Dept Biochem, IBPC, Paris, France; 5) Cologne Center for Genomics, Cologne, Germany; 6) LIFA, Münster Univ, Germany; 7) Univ Children's Hospital Zürich, Switzerland; 8) Dept Biochem, Charite Univ Hosp, Berlin, Germany.

In the cblF defect of vitamin B12 metabolism, transcobalamin-bound cobalamin is endocytosed into cells, but free cobalamin accumulates in lysosomes presumably because of defective lysosomal export. Accordingly, synthesis of methylcobalamin and adenosylcobalamin is deficient, leading to hyperhomocysteinemia and methylmalonic aciduria. Microcell-mediated transfer of wild-type human chromosomes into immortalized fibroblasts from a cblF patient increased the incorporation of label from [14C]propionate towards normal values in clones isolated after the transfer of chromosome 6. Using high-density SNP arrays, we performed homozygosity mapping in 12 unrelated patients with cblF disease and identified *LMBRD1* as a positional candidate gene on chromosome 6. *LMBRD1* encodes LMBD1, a 61.4-kDa protein with 9 putative transmembrane helices, which shows significant homology to the lipocalin membrane receptor LIMR. Four different frameshift mutations, leading to premature termination codons and down-regulation of the transcript, result in a loss-of-function of both *LMBRD1* alleles in the patients. 18 of the 24 disease chromosomes analysed carry the same mutation on a common haplotype of 1.34 Mb. Transfection of cblF patient fibroblasts with the LMBD1 wild-type construct rescued cobalamin coenzyme synthesis and function. EGFP-tagged LMBD1 colocalized with the late endosomal and lysosomal membrane marker LAMP1. This identifies *LMBRD1* as the gene for cblF and suggests that it codes for a lysosomal membrane transporter for cobalamin.

The Prader-Willi syndrome (PWS) snoRNAs and those encoded by marsupial *SNRPB* and *SNRPN* are paralogous with divergent evolution from a shared therian ancestor. *W. Zhu[1], P. B. Samollow[2], R. D. Nicholls[1,3]* 1) University of Pittsburgh, Pittsburgh, PA; 2) Texas A&M University, College Station, TX; 3) Children's Hospital of Pittsburgh, Pittsburgh, PA.

The mechanisms and selective forces underlying the evolutionary acquisition of genomic imprinting and imprinted genes are not well understood. Further, the timing and origins prior to or during the eutherian expansion for some imprinted genes of extant mammals is not known. This is exemplified by several intronless genes that are located in the human chromosome 15q11.2 region associated with PWS and their orthologs, which derived by retrotransposition or retroviral insertion. In contrast, the PWS-region gene encoding the SmN spliceosomal core subunit (*SNRPN*) originated through a gene duplication putatively in the ancestor of eutherian and metatherian mammals. This study sought to determine the hitherto unknown evolutionary origins of the five classes of box C/D small nucleolar RNA (snoRNA) genes found in the PWS domain. Uniquely in metatherians the paralogous *SNRPB* and *SNRPN* loci are adjacent genes and each contain novel snoRNA paralogs, *SNORD119B* and *SNORD119N*, in their respective fifth introns. *SNORD119B* orthologs also exist in eutherian mammals, a reptile, an amphibian, and some teleost fishes (variably in intron 5 or 6) but not in birds. Based on quantitative RT-PCR on RNA from a marsupial, *Monodelphis domestica, SNRPN* is expressed at higher levels in brain and lower levels in other tissues than *SNRPB*, as is true for eutherians. Surprisingly, *SNORD119N* levels are higher than *SNORD119B* levels in all tissues possibly from increased RNA stability due to 28S rRNA targeting. Using phylogenetic sequence comparisons, we propose an evolutionary pathway for the origins of each PWS snoRNA class from an ancestral *SNORD119N* gene. Most eutherian PWS genes (*SNRPN* and each snoRNA) thus arose evolutionarily by stepwise duplication and divergence from an ancestral *SNRPB-SNORD119B* locus present in the common ancestor of metatherian and eutherian mammals. Additionally, our data support the notion that snoRNA evolution can be labile, with duplication, transposition, and loss detected in different species.

Sequencing human-gibbon breakpoints of synteny. *L. Chen[1], S. Girirajan[1], T. Graves[2], T. Marques-Bonet[1], E. Mardis[2], E. E. Eichler[1]* 1) Department of Genome Sciences, Howard Hughes Medical Institute, University of Washington, Seattle, WA; 2) Genome Sequencing Center, Washington University, St. Louis, MO.

Gibbons offer an excellent phylogenetic link between the great apes and Old World monkeys. They exhibit an extensive karyotypic diversity with an increased rate of chromosomal translocations during the hominoid evolution. We examined, at base pair resolution, 25 synteny breaks encompassing 4.4 Mb of white-cheeked gibbon (Nomascus leucogenys, NLE) sequence with respect to the human genome for its contents, effects, and dynamic nature. About 50% of the breakpoints in human syntenic region fell within a repeat-rich region. A 1.5-2-fold enrichment of LINE, SINE, and LTRs was observed in the gibbon BACs and within a 20 kb breakpoint window compared to control gibbon WGS reads (~8 Mb). We also identified a 10-fold enrichment of L1PA4 elements and an increased abundance (9.5%) of segmental duplications with a majority of gibbon-specific duplications (89%) in our study. Further, segmental duplication-associated events including formation of paralogous genes and pseudogenes were observed in the vicinity of the breaks. However, no evidence of segmental duplication-mediated NAHR contributing to the gibbon human synteny breaks was apparent in our studies. Seven genes, involved in signaling pathways (*DEPDC4*, *GNG10*), phospholipid metabolism (*ENPP5*, *PLSCR2*), -oxidation (*ECH1*), cellular structure and transport (*HEATR4*), and transcription factors associated with gonadal functions (*GIOT1*) were disrupted due to synteny breaks in gibbons. Additionally, growth hormone cluster (*GH2*, *CSH1*, *CSH2*, and *CSHL1*) and genes involved in spermatogenesis (*ALMS1*) were also mutated in the vicinity of the breaks. An evolutionary analysis of coding sequences showed that the disrupted genes are affected by different selection pressures. Our study provides a catalog of molecular events at the synteny breaks in the white-cheeked gibbons and suggests its potential genetic effects contributing to the morphological and phenotypic uniqueness in gibbons.

Two separate mutations contributing to disease severity in an autosomal dominant Retinitis Pigmentosa (RP) family. *M. Gorin[1], A. Martinez[1], E. Spector[2], P. Chiang[2]* 1) Jules Stein Eye Institute, Department Ophthalmology, UCLA School of Medicine, Los Angeles, CA; 2) 2.UCD DNA Diagnostic Laboratory, Department of Pediatrics, UCD School of Medicine , Aurora, CO.

Retinitis pigmentosa (RP) is a group of inherited disorders affecting the photoreceptors or the retinal pigment epithelium (RPE) of the retina and leading to progressive visual loss. One approach for RP molecular diagnostics is to screen for known mutations in a CHIP-based platform. A second approach is to sequence the most common RP-related genes and proceed in a sequential fashion towards screening less common causative genes. We have employed an alternative approach by sequencing every coding exon from every known adRP gene. A total of 130 polymerase chain reactions (PCR) covering every coding exon from the 17 known adRP genes were performed and sequenced. In our first trial of this approach, we selected an adRP family with a clear autosomal dominant inheritance pattern. We identified a known mutation G56R in NR2E3 and a novel variant E160K in FSCN2 in the proband. The probands severely affected son has both variants, while another family member carrying only the G56R mutation is only exhibiting night blindness at the age of 73. The probands daughter, carrying only the E160K mutation, is asymptomatic, although she is still very young. The G56R mutation in NR2E3 reportedly accounts for 1-2% of adRP. The E160K variant in FSCN2 changes a highly conserved amino acid from an acid to a base that is likely to have an adverse effect on protein conformation and function. The potential modifier effect of the E160K mutation is suggested by the presence of both G56R and E160K mutations in the two most severely affected individuals. Others within this family are being ascertained to correlate clinical findings (including night blindness) with one or both of these mutations. These findings also highlight a potential limitation of both the CHIP-based and tiered analysis approaches towards RP that would have missed this potential modifier variant. It remains to be seen if we will find similar, potential disease modifier variants in other RP families through this comprehensive screening approach.

Analysis of mtDNA and Y-chromosome haplogroups in Mexican Mestizos and Amerindian groups. *I. Silva-Zolezzi[1], B. Z. Gonzalez-Sobrino[2], J. K. Estrada-Gil[1], A. Contreras[1], J. C. Fernandez[1], E. Hernandez-Lemus[1], L. Sebastian[1], F. Morales[1], R. Goya[1], C. Serrano[2], G. Jimenez-Sanchez[1]* 1) National Institute of Genomic Medicine, Mexico; 2) Anthropological Research Institute, UNAM, Mexico.

The Mexican population is mainly conformed by Mestizos, individuals with a genetic background consisting of Amerindian, European and African contributions. Genetic heterogeneity in Mexicans results from a complex demographic history that started with the peopling of North and Central America about 15,000 yrs ago, including the settlement of at least 60 different indigenous groups in Mexico, regional differences in admixture dynamics after colonization by Spaniards in the XVI century, epidemics and migration. Y chromosome-specific and mitcohondrial (mt) DNA polymorphisms are useful to help understand the genetic structure and history of human populations, due to their uniparental inheritance and lack of recombination. In order to refine the portrait of genetic variability derived from the Mexican Genome Diversity Project, we are characterizing maternal and paternal lineages participating in admixture. For this we included genotypic data from 163 mt SNPs and 123 Y chromosome SNPs present in the Illumina Human1M chip of 450 individuals, 300 mestizos from six states located in different regions: Northern, Central and Southern; and 150 individuals from different Amerindian groups (Tepehuanes, Zapotecos and Mayas). With this information, we are measuring genetic diversity using Fst and AMOVA analysis. Admixture analysis includes average and individual ancestral contribution estimates using autosomal SNPs. Initial results show that in our Mestizo sample, 88% of the mt haplogroups are Amerindian (A, B, C or D), and the rest includes European and African lineages. We have identified differences in proportions of each haplogroup in both Mestizos and Amerindians. Knowledege about the distribution of mt and Y-chromosome haplogroups in Mexican Mestizos and Amerindian groups, will generate valuable information to better understand genetic relationships between Mexicans and other Latin American populations. In addition, it may contribute to strengthen analysis in association studies of common complex diseases.

Genome-wide analysis of GxE: genotype by smoking effects on gene expression in the small airway of the lung. *J. G. Mezey[1], C. Gao[1], M. Butler[2], A. G. Clark[3], N. R. Hackett[2], T. P. O'Conner[2], R. G. Crystal[2]* 1) Biological Statistics (BSCB), Cornell Univ, Ithaca, NY; 2) Weill Medical College of Cornell University, New York, NY; 3) Molecular Biology and Genetics, Cornell Univ, Ithaca, NY.

We applied two methods for detecting genotype by environment (GxE) interactions in a genome-wide analysis of gene expression phenotypes: linear model based hypothesis testing and Bayesian mixture prior multiple regression. We applied these techniques to assess the degree to which smoking modulates genetic effects on gene expression in the small airway epithelium (SAE) of the lung, a cell population central to the pathogenesis of pulmonary disease. Genes expressed in the airway epithelium are known to be responsive to smoking and can also be affected by an individuals genotype, although the degree to which these factors interact at a genome-wide scale is currently unknown. Gene expression levels in the SAE cells obtained by fiberoptic bronchoscopy and brushing, were assayed using Affymetrix HG-U133 Plus 2.0 arrays in 106 healthy non-smokers, healthy smokers, and smokers with Chronic Obstructive Pulmonary Disease (COPD) of African, European, and Hispanic ancestry. Single nucleotide polymorphisms (SNPs) were also identified for these individuals using the Affymetrix 5.0 SNP array. We tested for significant genotype by smoking interactions for individual markers found within 25,000 bp of over 12,000 genes for which we had measured expression, accounting for effects of ancestry. Smoking has a dramatic impact on the gene expression profile, resulting in greater than 2-fold up- and down-regulation of over 150 probe sets. Even with the relatively small sample size, our analysis was able to identify highly significant SNP associations with SAE gene expression which are modulated by smoking. In addition, the Bayesian analysis was able to identify probable false-positives and GxE effects which were hidden by the structure of local linkage disequilibrium in the sample. These results are consistent with a genetic basis for differential responsiveness in SAE gene expression to smoking and may indicate important GxE relationships which impact the susceptibility to smoking-associated disease.

Leucoencephalopathy with megalencephaly, spongy degeneration and 17p deletion: A new disease? *M. Schiff[1], S. Drunat[2], S. Passemard[2], A. Aboura[2], F. Chalard[3], A. Verloes[2]* 1) Pediatric Neurology and Metabolic disease Dept, Robert Debré Hospital, Paris, France; 2) Genetics Dept, Robert Debré Hospital, Paris, France; 3) Pediatric Radiology Dept, Robert Debré Hospital, Paris, France.

This second child from non consanguineous parents was born at term by cesarean section for unexplained hydramnios. Birth parameters were at -1 SD. After minor neonatal feeding difficulties, she was investigated at 3 years of age for moderate and non progressive psychomotor retardation (with mainly speech delay) and dysmorphic features. Clinical findings included macrocephaly (head circumference +3 SD), contrasting with growth delay (weight -1 SD, height -2 SD), wide forehead, small nose and coarse facial features. There was no hepatosplenomegaly, no joints contractures and no signs of peripheral neuropathy. Basic biological findings were normal. Brain MRI disclosed bilateral and symmetric leucoencephalopathy with triventricular dilation, micro cysts in periventricular white matter and corpus callosum and Chiari type 1 malformation. There was no abnormal lactate or N-acetyl-aspartate peak on spectroscopy and urinary organic acids were normal, excluding Canavan disease. Activities of hexosaminidases A and B measured in leukocytes were normal, excluding GM2 gangliosidosis. CGH-array (Agilent 44K) revealed a large (2 Mb) subtelomeric deletion of the 17p region between nt 48539 and nt 2104702, thus from RPH3AL (rabphilin 3A-like, without C2 domains) to SMG6 (nonsense mediated mRNA decay factor). The deletion encompassed 38 genes, none of which known to be involved in leucoencephalopathy. The aspartoacylase gene (Canavan) is located centromeric to the deletion. Although the deletion could be coincidental with the cerebral phenotype, our patients presentation suggests that the 17pter region may contain a second locus for leucoencephalopathy with spongy degeneration. Inheritance could be dominant or recessive (with haploinsufficency by deletion for one allele, and loss-of-function point mutation for the second allele).

An unbalanced translocation between chromosomes 6p and 2p associated with Axenfeld-Rieger anomaly, hearing loss, developmental delay and distinct facial dysmorphism. *D. A. S. Batista[1,2], F. Li[4], I. Maumenee[3], T. Wang[4]* 1) Kennedy Krieger Institute, Baltimore, MD; 2) Departments of Pathology; 3) Ophtalmology; 4) Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD.

Chromosome rearrangements have long provided clues that allowed mapping genes for genetic disorders. We describe a 14-year old boy with severe growth and global developmental delays, autistic behaviors, congenital hearing impairment and notable dysmorphic features including prominent eyes, down-slanting palpebral fissures, broad nasal bridge, hypoplastic maxilla, high arch palate, long and thin digits. He has reduced muscle bulk, bilateral planovalgus deformity with tight heel cord, persistent hamstring contracture, mild scoliosis and kyphosis. Ophthalmology examination revealed complex malformations of anterior segment including anomalies of anterior chamber angle, prominent Schwalbe line, iris hypoplasia, tear-drop shaped pupil and high myopia on the right, and congenital glaucoma, consistent with Axenfeld-Rieger anomaly (ARA). Normal electroretinogram was documented at age 13 years. Brain MRI studies revealed dysgenesis of corpus callosum, enlargement of ventricles, decreased volume of cerebral white matter and patchy distribution of T2 hyperintensity in the white matter surrounding lateral ventricles. High-resolution karyotype and array CGH with 4200 BAC clones (BlueGnome) showed a de novo unbalanced translocation between the short arms of chromosomes 2 and 6 resulting in 8MB gain from 2p25.1 to pter and 6MB loss from 6p25.1 to pter. The trisomic genes at 2p (n=23) include SOX11, a transcription factor that is important in CNS development. The monosomic genes at 6p (n=40) include FOXC1, a member of the forkhead gene family. Mutations or haploinsufficiency of FOXC1 are associated with defects in anterior segment of the eye. We conclude that the 6p25.1-pter deletion involving FOXC1 is responsible for ARA in this patient. Further study of this unbalanced translocation should expand the phenotypic spectrum of 6p25 microdeletion syndrome and implicate essential genes at 6p25 and/or 2p25 in normal brain and eye development in humans.

Later onset mitochondrial myopathy without hepatic disease caused by mutations in *DGUOK*. *D. Dimmock[1], S. Zhang[2], L.-J. C. Wong[2]* 1) Pediatrics, Medical College of Wisconsin, Milwaukee, WI; 2) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Background Deoxyguanosine kinase (DGUOK) deficiency was originally described as the cause of an infantile onset hepatocerebral mitochondrial disease. The key features of this disorder include significant hepatic failure with nystagmus and hypotonia. Mitochondrial DNA studies reveal significant mitochondrial DNA depletion in the affected tissues. Subsequently it has been shown that the same mutations in this gene may present with isolated acute liver failure without cerebral or hepatic involvement. Conversely, Thymidine Kinase 2 (TK2) has been shown to cause a predominantly myopathic form of mitochondrial DNA depletion with significantly elevated creatinine kinase. **Case** Here we present a juvenile patient with cardiomyopathy, cyclic neutropenia, exercise intolerance and significant hypotonia. He does not have liver failure but did have abnormal mitochondrial electron transport chain activity with profound mitochondrial DNA depletion on muscle biopsy. Mutations were not found in *TK2* but instead we found in trans, two previously published mutations in *DGUOK*. **Conclusions** This case report expands the clinical spectrum of DGUOK deficiency. It suggests that, in patients with myopathic mitochondrial DNA depletion, if mutations are not found in *TK2*, other genes should be evaluated.

Accelerated genetic drift on chromosome X during the human dispersal out of Africa. *A. Keinan[1,2], J. Mullikin[3], N. Patterson[2], D. Reich[1,2]* 1) Department of Genetics, Harvard Medical School, Boston, MA; 2) Broad Institute, Cambridge, MA; 3) National Human Genome Research Institute, NIH, Bethesda, MD.

Comparing genetic variation on chromosome X and the autosomes enables a multi-locus study of differences in the demographic histories of men and women. We have recently identified subsets of 150,000 SNPs from phase 2 of HapMap that are free of ascertainment bias and usable for learning about history (Keinan et al. Nature Genetics 2007). We have now extended these data to chromosome X, offering the first large-scale data set that allows accurate comparison of chromosome X and the autosomes. We also generated a complementary data set by aligning more than a billion bases of sequence from individuals of known ancestry and estimating the average time since genetic divergence. Three independent lines of evidence suggest that during the dispersal of modern humans out of Africa, chromosome X experienced much more genetic drift than is expected from the pattern on the autosomes: (1) Chromosome X exhibits unexpectedly high allele frequency differentiation between Africans and non-Africans, but not amongst non-Africans. (2) We observed many more SNPs of high derived allele frequency on chromosome X in non-Africans, but not in Africans, compared with what is expected from the autosomes. (3) We showed a reduction amongst non-Africans in the time since the most recent common ancestor for chromosome X loci compared with what is expected from the autosomes. All lines of evidence account for differences in effective population size and mutation rate between the two parts of the genome. The results cannot be explained by known episodes of human history or natural selection, and suggest that men participated more than women in the dispersal out of Africa. A parsimonious explanation is that after an initial founder population was formed by migration of both men and women, much of its gene pool was replaced by subsequent male migrants, from whom non-African populations inherit most of their diversity. These results have methodological implications for human population and medical genetic studies, where it is currently commonly assumed that chromosome X and the autosomes reflect the same history.

The FTO/obesity associated locus and dietary intake in children. *G. Davey Smith[1], P. Emmett[2], T. Frayling[3], I. Rogers[4], A. Hattersley[3], M. McCarthy[5], N. Timpson[1,5]* 1) MRC CAiTE Centre, Bristol University, UK; 2) Bristol University, UK; 3) Peninsula Medical School, UK; 4) Brighton University, UK; 5) Oxford University, UK.

Variation in the fat mass/obesity associated gene (FTO) is associated with fat mass, body mass index (BMI), and obesity. We aimed to assess the role that appetite plays in this association by using detailed dietary reports from the Avon Longitudinal Study of Parents and Children. Analyses assessed possible associations between variation at the FTO locus and a range of micro and macro-nutrients, taking into account the bias often found within dietary report data when assessing factors related to BMI. We also assessed associations between FTO and dietary intake independent of BMI in order to test the hypothesis that FTO may be influencing appetite directly as opposed to indirectly via BMI and altered intake requirement. Within a sample from which those underreporting food intake were removed, relationships between a single nucleotide polymorphism characterising the FTO signal (rs9939609) and dietary variables were found; total energy, energy from food, saturated fat, monounsaturated fat, polyunsaturated fat all $p<=0.009$ and trans-fatty acid $p=0.01$ at age 10-11. In efforts to account for increases in basal metabolic rate resulting from greater BMI, we adjusted for BMI and found that association remained (and was of similar magnitude) for total energy from all food (ratio of geometric means 1.008, SE 0.0037, $p=0.03$), total fat (ratio of geometric means 1.012, SE 0.005, $p=0.02$) and saturated fat (ratio of geometric means 1.012 SE 0.007, $p=0.05$). For fat and energy consumption these can be thought of as total daily fat consumption approximately 1.5g/day difference per allele and total daily energy consumption approximately 25kj/day difference per allele. There was no difference by genotype in the percentage of total energy (kcal) consumed as fat, carbohydrate and protein. Taken together, these associations suggest that individuals carrying minor variants at rs9939609 were consuming more fat and total energy, and that this was not simply dependent upon them having higher average BMI levels.

Participation in genome-wide association studies using electronic medical records: trust is key. *S. M. Fullerton[1], S. B. Trinidad[1], J. M. Bares[1], G. P. Jarvik[2], E. B. Larson[3], W. Burke[1,2,3]* 1) Medical History & Ethics, University of Washington, Seattle, WA; 2) Medical Genetics, University of Washington, Seattle, WA; 3) Center for Health Studies, Group Health Cooperative, Seattle, WA.

Genetic research, particularly as conducted on a genome-wide population-based scale with links to medical records, poses significant new challenges for informed consent, management and protection of personal information, and the return of research findings to groups and individuals. As part of the NHGRI-sponsored eMERGE (electronic Medical Records and Genomics) network, we carried out focus group discussions with members of the Group Health Cooperative (GHC), a non-profit health maintenance organization. Ten focus groups were held with distinct classes of GHC stakeholders: current genetic research participants, surrogate decision makers of deceased or incapacitated research participants, and members not currently participating in research. Each group met for approximately 2 hours to provide their perceptions and opinions about genomic research, what should be covered in the informed consent process, and the risks and benefits of participation. Discussions were audio recorded, transcribed, and analyzed to identify topics and themes of significance. In general, participants reported a high degree of willingness to participate in genome-wide association studies and agreed that appropriately de-identified genetic and linked medical record data could be shared for broad research benefit, as long as the possibility of such sharing was outlined in the initial consent. Their reported confidence in the value of such research was directly tied to their faith in GHC as a reputable institution that would provide strong stewardship of their personal data. In contrast, most participants were uncomfortable with the possibility that their personal data might be shared with for-profit entities. Focus group participants also expressed the expectation that clinically relevant results would be returned wherever possible. Results of the focus group discussions will be used to inform a year-long internal consensus development process aimed at informing GHC policies for conduct of future genetic investigations.

Risk of Interferon-gamma (INF-) gene polymorphism (+874 A/T) to age related cataracts. *M. MANNE[1], S. G. Bhagyalaxmi[1], R. Sireesha[1], D. V. Raje[2], S. Kiran[2], M. Vidyavathy[3], K. R. Reddy[3], G. Sridhar[4], T. Nagaraju[4], T. Padma[1], M. Manne[1]* 1) Department of Genetics, Osmania University; 2) Ocimum Biosolutions Pvt. Ltd; 3) Sarojini Devi Eye Hospital & Inst. of ophthalmology; 4) Department of Zoology, Osmania University, Hyderabad, India.

Low molecular weight molecules that act as UV filters protecting retina are derived from tryptophan catabolism via, kynurenine pathway by the enzyme indoleamine-2, 3, dioxygenase (IDO). This enzyme is induced mainly by Interferon IFN in infections, inflammations etc. IFN- gene shows polymorphism that may be differentially related with induction of IDO. We studied 426 cataract cases (102 Nuclear-NC, 99 cortical-CC, 94 posterior subcapsular-PSC and 131 mixed-MC type) and 123 controls to predict risk for IFN- genotypes. In all types of cataracts there was preponderance of females and non obese subjects with later onset in NCs and early onset in PSCs. While frequencies of IFN- genotypes did not differ significantly in NC and CC, a higher frequency of AT heterozygotes was observed in PSC (50.0%) and MC (48.1%) patients as compared to controls (34.9%;). Heterozygous females showed high frequency in PSC (53.8%) and MC (44.5%) cases and that of AA homozygotes in CC (61.3%) cases compared to controls (AT-32.1%, AA-54.7%. There was high risk for AT hetrozygotes for PSC (OR-1.86; CI-1.07-3.21; p0.02) and MC (OR-1.72; CI-1.04-2.85; p0.03) and low risk for AA homozygotes for MC (OR-0.44; CI-0.27-0.74; p0.001. Between the cataract types MCs differed significantly from other types in the distribution of IFN- polymorphism (MC vs. NC x2-10.90, 2d.f. p-0.004; MC vs. CC x2 -12.99, 2d.f. p- 0.001 and MC vs. PSC x2-6.04, 2d.f. p-0.04). Considering gender variation while both the sexes showed significant difference when CCs were compared to MC type (males: x2-6.29, 2d.f. p- 0.04; females: x2-7.68, 2d.f. p-0.02) in PSCs the difference was significant only in females(x2-6.93, 2d.f. p-0.03). These results showed high risk for heterozygotes of IFN genotypes for PSCs and MCs. Study of these individuals for variation in the regulation of IDO seems worth pursuing to have better understanding of the process of cataractogenesis.

Genome-Wide Association Study Reveals a Novel Locus for Male Pattern Baldness. *J. B. Richards[1,2], X. Yuan[3], F. Geller[4], D. Waterworth[3], V. Bataille[2], D. Glass[2], K. Song[3], G. Waeber[5], P. Vollenweider[5], B. Walters[4], U. Thorsteinsdottir[4], A. Kong[4], H. Stefansson[4], K. Stefansson[4], T. D. Spector[2], V. Mooser[3]* 1) McGill University, Montreal, QC, Canada; 2) King's College London, London, United Kingdom; 3) Genetics Division, GlaxoSmithKline, King of Prussia, Pennsylvania, USA; 4) deCODE Genetics, Reykjavik, Iceland; 5) CHUV University Hospital, Lausanne Switzerland.

Objective: Androgenic alopecia is a highly heritable polygenic disorder of considerable social significance, whose genetic architecture remains to be described. We conducted a genome-wide association study for androgenic alopecia **Methods:** A genome-wide association study was performed assessing 370,102 single nucleotide polymorphisms (SNPs) from the Affymetrix gene chip human mapping 500k array using standard protocols in 578 male cases (defined as Hamilton grade alopecia V-VII prior to age 65) and 547 male controls (defined as Hamilton grade I-II before age 75). The lead SNPs from novel loci achieving a p-value of 10^{-5} were tested for replication in two independent cohorts involving 1,100 men from the United Kingdom and Iceland, phenotyped for androgenic alopecia and hair loss, respectively. Additionally, this SNP was tested for association with hair loss in 878 women from Iceland. **Results:** The previously replicated locus near AR on the X chromosome achieved genome-wide significance ($p = 5.0 \times 10^{-11}$). 26 SNPs achieved a p-value of 10^{-5} from a novel locus on chromosome 20 in the discovery genome-wide association scan. The lead SNP from this locus was strongly associated with androgenic alopecia ($p = 3.2 \times 10^{-10}$) in the discovery cohort and was also associated with androgenic alopecia in 366 men from the British TwinsUK cohort ($p = 1.5 \times 10^{-3}$) and hair loss in 734 men from the Icelandic cohort ($p = 6.1 \times 10^{-3}$). The combined odds ratio for androgenic alopecia in men for all cohorts was 1.6 ($p = 6.7 \times 10^{-13}$). Additionally, this SNP was associated with hair loss in women from Iceland ($p = 0.01$). **Interpretation:** Our findings demonstrate that a novel locus on chromosome 20 is strongly associated with androgenic alopecia.

Genetic Associations with Coronary Disease from Integer and Lipid-Weighted Genetic Risk Score Algorithms for Six Lipid Metabolism Genes. *B. D. Horne[1,2], J. F. Carlquist[1,3], N. J. Camp[2], C. P. Mower[1], J. J. Park[1], J. B. Muhlestein[1,3], J. L. Anderson[1,3]* 1) CV Dept, Intermountain Med Ctr, Murray, UT; 2) Genet Epi Div, Univ Utah, SLC, UT; 3) Cardiol Div, Univ Utah, SLC, UT.

Genetic associations with common, complex phenotypes suggest small risk effects from most single nucleotide polymorphisms (SNPs). A polygenic genetic risk score (GRS) aggregates information from multiple SNPs in many genes. This study applied two GRS approaches to tagging (t) SNPs from 6 lipid genes for associations with coronary artery disease (CAD). To select tSNPs, each genes promoter, exons, exon/intron boundaries, and 3UTR were scanned in 50 normal controls. SNPs were genotyped in 339 normals to select tSNPs. A total of 38 tSNPs were selected (*CETP*: 11, *LIPC*: 11, *LCAT*: 3, *SR-B1*: 4, *LPL*: 7, *ApoF*: 2). CAD patients (n=1,020), non-CAD angiography patients (n=552), and untreated population normals (n=570) were genotyped. A simple integer GRS (IGRS) was calculated by summing the genotype score (-1, 0, 1) based on an additive model (genotype risk was determined from lipid values in untreated normals). A weighted GRS (WGRS) integrated SNP associations with lipid parameters. For IGRS and WGRS, a separate GRS was computed for LDL-C, HDL-C, and TG. Among normals, association was found for 7 SNPs with LDL-C, 5 SNPs with HDL-C, and 4 SNPs with TG. Compared to normals, no CAD associations were found for $IGRS_{LDL}$ (p-trend=0.13), $IGRS_{HDL}$ (p-trend=0.85), or $IGRS_{TG}$ (p-trend=0.47); results were similar for cases vs. non-CAD patients (p-trend=0.13, 0.59, 0.38, respectively). For WGRS in cases vs. normals, trends were found for $WGRS_{HDL}$ (OR=0.91/quartile, p-trend=0.07) and $WGRS_{TG}$ (OR=1.09/quartile, p-trend=0.08) but not $WGRS_{LDL}$ (OR=1.03/quartile, p-trend=0.56), while for cases vs. non-CAD patients association was found for $WGRS_{HDL}$ (OR=0.91/quartile, p-trend=0.041; adjusted: OR=0.89/quartile, p-trend=0.019) but not $WGRS_{TG}$ (OR=1.04/quartile, p-trend=0.39) or $WGRS_{LDL}$ (OR=0.94/quartile, p-trend=0.16). Weighted but not integer GRS variables were associated with clinically-relevant genetic contribution to CAD. Polygenic GRS metrics combining many related low-effect SNPs may enable detection of genetic associations.

High throughput oncogene mutation profiling using MALDI-TOF Mass spectrometry. *A. O. H. Nygren, G. Hogg, R. M. McCullough, D. van den Boom, M. Ehrich* SEQUENOM Inc., San Diego, CA.

In the current era of genomics, cancer research has made great strides in dissecting the molecular basis of individual tumors. In this context multiple mutations contribute to a number of genetic subtypes in histologically homogeneous tumor class. To obtain an accurate molecular classification of large sample sets a flexible high-throughput solution is needed that can easily adapt to newly discovered mutation as well as accurately detect small amount of mutated DNA species, which may impact therapy selection or outcome determination. We have developed 20 multiplex reactions comprising more than 200 mutations in 17 oncogenes, ranging from single base changes to insertions and deletions of dozens of nucleotides. The assay relies on SEQUENOMs genotyping technology, which provides a substantial advantage over previous multiple base extension assays. For verification of this assay we have screened the NCI-60 set of cancer cell lines. The NCI-60 is a set of different human cancer cell lines derived from diverse tissues; brain, blood and bone marrow, breast, colon, kidney, lung, ovary, prostate and skin. Here we show that MALDI-TOF mass spectrometry assay is an ideal tool for high-throughput screening of these types of genetic lesions. By combining the robust SEQUENOM genotyping technology biochemistry with the sensitivity and accuracy of MALDI-TOF MS detection, an exact ratio is obtained for each pair of wild type versus mutated allele enabling assessing the impact of each individual mutation. In addition, by screening for up to 12 different mutations in each plex, we show that this assay requires a minimum of total sample DNA and many samples can be processed in parallel.

Is VEGF a modifier of the cardiovascular phenotype of the 22q11 microdeletion syndrome? *G. M. Repetto[1], J. F. Calderón[1], M. L. Guzmán[1], A. Puga[1], C. P. Astete[2], M. Aracena[2], M. Arriaza[3], T. Aravena[4], P. Sanz[5]* 1) Dept Genetics, Clínica Alemana- Universidad del Desarrollo, Santiago; 2) Hospital Luis Calvo Mackenna, Santiago; 3) Hospital Gustavo Fricke, Viña del Mar; 4) Complejo Hospitalario Dr. Sótero del Río, Santiago; 5) Hospital Clínico Universidad de Chile, Santiago, Chile.

Chromosome 22q11 microdeletion syndrome (del22q11) is a common identifiable cause of congenital heart disease (CHD). It is estimated that 50-75% of patients have CHD, mostly involving the cardiac outflow tract. The majority of patients share a common 3 Mb deletion, but the cause of the phenotypic variability is unknown. Data on animal models of the deletion, additionally deficient in VEGF, and an association study of promoter polymorphisms in patients with del22q11 suggest a role for VEGF as a modifier of the cardiovascular phenotype of this syndrome (Stalmans et al Nat Med 2003). We evaluated VEGF promoter polymorphisms -2578 AC, -1154 AG and -634CG, known to downregulate VEGF expression, in 112 patients with del22q11 and their parents. Half of the patients had CHD and the remainder had normal cardiac anatomy. Allelic and genotypic frequencies were compared by ^{2} between patients with del22q11 with or without CHD. No statiscally significant difference was observed. Transmission disequilibrium testing (TDT) was also performed, and the results showed no evidence of excess transmission of any of these polymorphisms. Our findings do not support a role for VEGF promoter polymorphisms as modifiers of the presence of congenital heart disease in Chilean patients with del22q11. Funded by Fondecyt-Chile, Grant 1061051.

A Developmentally-Imposed Fixed Alteration in Cellular Identity Contributes to Pathogenesis in Marfan Syndrome (MFS). *M. E. Lindsay[1,2], D. C. Loch[1], Y. Chen[1], H. C. Dietz[1,3]* 1) Institute of Genetic Medicine; 2) Division of Pediatric Cardiology; 3) HHMI, Johns Hopkins Hospital, Baltimore, MD.

Aortic root aneurysm is the major cause of mortality in MFS, a disorder caused by deficiency of fibrillin-1. Many features of MFS manifest failed matrix regulation of TGF, and can be prevented in mouse models by TGF antagonists including the angiotensin II (AngII) type 1 (AT1) receptor blocker losartan that reduces expression of TGF ligands, receptors, and activators such as thrombospondin-1 (TSP1). Cultured vascular smooth muscle cells (VSMCs) derived from the aortic root of patients show abnormally high expression of AT1, TSP-1, and selected VSMC markers (smooth muscle actin and calponin), but fail to express markers of terminal VSMC differentiation such as smoothelin. These cellular phenotypes are more indicative of myofibroblasts than VSMCs. In theory, TGF could impose this phenotypic switch via a reversible change from VSMCs to a less mature state or a developmentally-fixed transformation via endothelial-to-mesenchymal transition (EnMT). This question was addressed directly by lineage tracing studies using the Rosa26 reporter allele that only expresses lacZ upon Cre-mediated removal of a buffer sequence. Pairing of Rosa26 with an endothelial cell-specific Cre driver will mark blue any cell that is or ever derived from an endothelial cell. Both WT and MFS mice showed positive aortic valve and endothelial cells, but positive cells within the aortic media were unique to MFS. Pathologic EnMT was restricted to the proximal aortic root, the region of unique predisposition for aortic enlargement in MFS and the only aortic segment that is normally populated by the secondary heart field. In this light, MFS transitions from good cells being stimulated to behave poorly during development due to an altered matrix environment to bad cells being themselves as a result of convergent predispositions imposed by cellular ontogeny and genetic perturbation. This novel paradigm will likely prove relevant to other genetic conditions and has implications for therapeutic intervention; based on these data pharmacologic inhibitors of EnMT are being explored in mouse models of MFS.

Undifferentiated human adipose-derived stromal cells express human muscle proteins and improve clinical features in SjL dystrophic mice. *N. M. Vieira[1], C. R. Bueno Junior[2], V. Brandalise[1], L. V. Moraes[4], E. Zucconi[1], M. Secco[1], M. F. Suzuki[3], M. M. Camargo[4], P. Bartolini[3], P. C. Brum[1], M. Vainzof[1], M. Zatz[1]* 1) Human Genome Research Center, Biosciences Institute, University of Sao Paulo;; 2) School of Physical Education and Sport, University of São Paulo;; 3) Biotechnology Department, National Nuclear Energy Commission-IPEN-CNEN, São Paulo;; 4) Department of Immunology, Instituto de Ciências Biomédicas, University of São Paulo - Brazil.

Limb-girdle muscular dystrophies (LGMD) are a heterogeneous group of disorders characterized by progressive degeneration of skeletal muscle caused by the absence or defective muscular proteins. The murine model for Limb-Girdle Muscular Dystrophy 2B (LGMD2B), the sjl mice, carry a deletion in the dysferlin gene that causes a reduction in the protein levels to 15% of normal. The mice show muscle weakness that begins at 4-6 weeks and is nearly complete by 8 months of age. The possibility to restore the defective muscle protein and improve muscular performance by cell therapy is a promising approach for the treatment of LGMD or other forms of progressive muscular dystrophies (PMD). Here we have injected human adipose stromal cells (hASCs) in the sjl mice, without immunosupression, aiming to assess their ability to: engraft into recipient dystrophic muscle after systemic delivery; form chimeric human/mouse muscle fibers; express human muscle proteins in the dystrophic host and improve muscular performance. We show for the first time that hASCs are not rejected after systemic injection even without immunosupression, are able to fuse with the host muscle, express a significant amount of human muscle proteins and improve motor ability of injected animals. These results may have important applications for future therapy in patients with different forms of muscular dystrophies.

Pierre Robin syndrome: report of a new case. *J. SANDRA GABRIELA[1], H. LAURA[2], G. DAVID OMAR[3], H. EDUARDO[4]* 1) NEUROPSICOGIA, INSTITUTO NACIONAL DE REHABILITACION, MEXICO D.F; 2) AUDIOLOGIA, INSTITUTO NACIONAL DE REHABILITACION, MEXICO D.F; 3) INSTITUTO NACIONAL DE REHABILITACION, MEXICO D.F; 4) FACULTAD DE ESTUDIOS SUPERIORES IZTACALA, UNAM, BIOLOGIA.

Pierre Robin syndrome it corresponds to a type of the ca syndromes craneofaciales. Prevalencia is 1:8500 new born, and de 80%, associates to specific syndromes. Their inheritance recessive autosomica, with a bound variant to X with heart malformations and foot Bot. Pierre Robin sequence (posterior U-shape cleft palate, glossoptosis, retrognathia) (PRS) is a frequent and heterogeneous neonatal condition of obscure origin. Orodigestive and cardiorespiratory functional disorders are very frequent in PRS and that these functional disorders, as well as anatomical and embryological data, argue for the involvement of brainstem dysfunction in the pathogenesis of some cases of isolated PRS. Prenatal and neonatal brainstem dysfunction as a neuroembryological hypothesis to explain the onset of some cases of Pierre Robin sequence. We present a new case: female mexican child 5-year-old, is the first child of young non consanguineous parentes, obtaind termino. She presented palatine fissure. That presents inconvenience of language, characterized by being found to level of dissyllables, basing its communication with signs and carrying to object desired. Uncle paternal grandfather with Down syndrome. EEG normal. Three years six months average mental age.

Identification of muscular dystrophy (MD) linkage regions and candidate genes. *K. K. McDonald, K. Crooks, J. Rimmler, J. Stajich, T. Jafarov, A. E. Ashley-Koch, M. A. Hauser* Center for Human Genetics, Duke University Medical Center.

Muscular dystrophy (MD) consists of a heterogeneous group of disorders that are characterized by progressive weakening and degeneration of skeletal muscle. Although multiple genes have been identified for autosomal dominant MD, they are only causal for a small proportion of cases. Therefore, many causative genes remain to be identified for inherited forms of MD. In this study, whole genome linkage analysis was performed with the Illumina Linkage IV Panel, containing over 6,000 SNPs, for four families with autosomal dominant limb-girdle muscular dystrophy (LGMD) or scapuloperoneal muscular dystrophy (SPMD). In each family, a single linked region was identified, and these regions were subjected to microsatellite fine mapping in order to further reduce the linked intervals. The minimal candidate intervals include a 14 cM region on chromosome 15 for LGMD family 383, a 21.2 cM region on chromosome 21 for LGMD family 1767, and a 30.9 cM region on chromosome 8 for LGMD family 2692. For SPMD family 2277, the linked region spans 16.8 cM on chromosome 3. The maximum nonparametric LOD score achieved for each region was 3.38, 3.30, 2.05, and 2.70 respectively. The number of positional candidate genes varies greatly among the families, from 33 genes in the region of interest for family 383 to greater than 100 genes for SPMD family 2277. We have prioritized these genes for further investigation based on expression pattern and function. Among the best functional candidate genes are talin-2 in family 383 and collagen 6A 1 and 2 in family 1767. We are currently sequencing these and other candidates. The identification of novel variants responsible for muscular dystrophy will provide immediate benefit for individuals in the families under study, by making possible genetic testing and counseling, in addition to enhancing the basic knowledge of muscle biology and the genes required for normal muscle function.

Cytosine methylation profiling of cancer cell lines suggest that PRC2 target methylation is a common event in cancer. *M. Ehrich[1], J. Turner[1], P. Gibbs[2], L. Lipton[2], M. Giovanneti[2], C. Cantor[1], D. van den Boom[1]* 1) Sequenom, Inc., San Diego, CA; 2) Ludwig Institute for Cancer Research, Royal Melbourne Hospital, Parkville, VIC, Australia.

It has been shown that a set of genes with binding sites for the polycomb repressive complex 2 (PRC2) in their promoter region can be epigenetically silenced in embryonic stem cells to maintain pluripotency. PRC2 involvement has been shown for colon cancer, but it is not known if these findings are applicable to other cancer types. We used a set of more than 400 cancer related genes for DNA methylation profiling in 59 cancer cell lines derived from 9 different types of cancer. We validated our cancer cell lines findings in a set of colon cancer and adjacent normal tissue samples. We confirmed more than 85% of genes with altered methylation in the cell lines to be differentially methylated in the clinical specimen. The set of examined genes included 70 that are known to be targets for PRC2. We were able to identify PRC2 target methylation in multiple forms of cancer. In six out of seven tumor types we find the fraction of hypermethylated genes to be enriched for those with PRC2 binding sites in the promoter region. These results suggest that PRC2 target silencing is a common event in cancer. The only tumor type with no such enrichment is melanoma, a cancer type with large environmental component that retains a high degree of differentiation. Our methylation data provides an ideal starting point for genome wide methylation research to identify all differentially methylated genes. The results can be combined with existing large scale datasets to develop an approach that integrates epigenetic, transcriptional and mutational findings.

A combination of spinal muscular atrophy carrier testing and linkage analysis can be used in prenatal testing when CVS is contaminated with maternal cells. *T. Prezant[1], M. Glicksberg[2, 3], T. Tran[1], L. Cowan[4, 5]* 1) University Children's Genetics Laboratory, a Division of ProGene, Glendale, CA; 2) El Camino Real High School, Woodland Hills, CA; 3) Brandeis University, Waltham, MA; 4) Good Samaritan Hospital, Los Angeles, CA; 5) Harbor UCLA Medical Center, Torrance, CA.

A family presented for prenatal diagnostic testing for Spinal Muscular Atrophy (SMA) Type I after molecular identification of the homozygous absence of *SMN1* exons 7 and 8 in their first child, who was affected. While the chorionic villus sample (CVS) was growing in culture, the parents were tested for carrier status by a new semi-quantitative SMA carrier test, which utilizes gene-specific PCR to determine dosage of *SMN1* exon 7. We determined that both parents are one-copy carriers. Diagnostic testing of the cultured CVS showed the presence of *SMN1* in the current pregnancy, and carrier testing revealed one-copy dosage of *SMN1* exon 7 in the fetal sample. Because we detected maternal cell contamination (MCC) for several microsatellite markers, these results were inconclusive. However, since we targeted polymorphic markers in the *SMN1* region on 5q13 for MCC analysis and included DNA from the affected sibling and both parents as controls, we essentially performed linkage analysis concurrent with the SMA diagnostic tests. Initial results showed that the fetus shares only one of the at-risk haplotypes with the affected sibling, supporting the findings from carrier testing. Expanded linkage analysis allowed us to determine that the fetus most likely inherited the normal paternal allele and the *SMN1*-deleted maternal allele, with minor MCC contributed by the normal maternal allele. Although the presence of the normal paternal allele suggested that at worst the fetus would be an SMA carrier, the family was offered and chose to have repeat testing on cultured amniocytes. These results confirmed the predicted SMA carrier status of the fetus. In this family, results were informative despite the occurrence of MCC. The combination of carrier testing and concurrent linkage analysis can provide early, accurate prenatal diagnostic testing for at-risk couples despite the possible presence of maternal cell contamination.

Mutations in *ZFP57* are associated with hypomethylation of multiple imprinted loci in patients with Transient Neonatal Diabetes (TND1). *I. K. Temple[1,2], J. Callaway[1,3], S. Marks[3], H. White[4], C. Acerini[5], S. Boonan[6], P. Dyanikli[7], H. Firth[8], J. Goodship[9], A. Haemers[10], J. Hahnemann[11], O. Kordonouri[12], A. Masoud[13], E. Oestergaard[14], J. Storr[15], S. Ellard[16], A. T. Hattersley[16], D. O. Robinson[1, 3], D. J. G. Mackay[1,3]* 1) Div of Hum Genet, Univ Southampton, Southampton, UK; 2) Acad Unit of Gen Med, Wessex Clin Genet Service, Southampton, UK; 3) Wessex Reg Gen Lab, Salisbury, UK; 4) Nat Gen Ref Lab, Salisbury, Uk; 5) Dept of Paed, Univ Cambridge, UK; 6) Gen Counsel Clin, Kennedy Centre, Glostrup, Denmark; 7) American Hosp, Istanbul, Turkey; 8) Dept of Med Genet, Addenbrooke's Hosp, Cambridge, UK; 9) Instit of Hum Genet , Newcastle Univ, UK; 10) Dept of Int Med-Endo, Maria Hosp, Nord Limburg, Belgium; 11) Med Gen Lab Cent, Kennedy Centre, Glostrup, Denmark; 12) KinderKrankenhaus auf der Bult, Hannover, Germany; 13) Child Services, Northwick Park Hosp, Middlesex, UK; 14) Dept Clin Genet, Nat Univ Hosp Rigshospitalet, Copenhagen, Denmark; 15) Dept Paed, Cumberland Inf, Carlisle, UK; 16) Instit Biomed Clin Sci, Penninsular Med Sch, Exeter, UK.

Transient neonatal diabetes (TND1) is a well recognised disorder of imprinting. 20% of cases have loss of methylation at the TNDDMR resulting in overexpression of *PLAGL1*. We have previously shown that some patients have a variable pattern of mosaic DNA hypomethylation at multiple imprinted loci. Clinical features include variable combinations of macroglossia, umbilical hernia, learning difficulties, and heart defects in addition to TND. Recurrence in sibs has been recently reported. By genome wide SNP genotyping in 6 consanguineous families, we identified a shared 15Mb region of homozygosity at 6p22. Sequencing of *ZFP57*, a zinc-finger transcription factor expressed in early development, identified homozygous mutations in all six probands, while no sequence alterations were found in any control chromosomes. A further non consanguineous proband was identified who was a compound heterozygote. We conclude that this novel syndrome, which has variable epigenetic and clinical characteristics, is an autosomal recessive imprinting disorder. This is the first description of a heritable global imprinting disorder compatible with human life.

Gene expression analysis of otosclerosis stapes footplates. *M. Ealy[1], W. Chen[1], G. Ryu[2], J. Yoon[2], D. Welling[3], M. Hansen[1], A. Madan[2,4], R. Smith[1]* 1) Molecular Otolaryngology Research Laboratories, Department of Otolaryngology, University of Iowa, Iowa City, IA; 2) Neurogenomic Research Laboratory, Department of Neurosurgery, University of Iowa, Iowa City, IA; 3) Department of Otolaryngology-Head and Neck Surgery, Ohio State University, Columbus, OH; 4) Institute for Systems Biology, Seattle, WA.

Otosclerosis is a complex disease that results in a common form of conductive hearing loss due to impaired mobility of the stapes. Stapedial motion becomes compromised secondary to invasion of otosclerotic foci into the stapedio-vestibular joint. Although environmental factors and genetic causes have been implicated in this process, the pathogenesis of otosclerosis remains poorly understood. To identify genetic contributors to otosclerosis we completed a microarray study of otosclerotic stapedial footplates. Because most stapes surgery is now done by laser-assisted stapedotomy, the number of pathological specimens was limited to nine. These stapes footplate samples and seven stapes footplates from control patients were used in the analysis. One-hundred-and-ten genes were found to be differentially expressed in otosclerosis samples. Ontological analysis of differentially expressed genes in otosclerosis provides evidence for the involvement of a number of pathways in the disease process that include interleukin signaling, inflammation and signal transduction, suggesting that aberrant regulation of these pathways leads to abnormal bone remodeling. Currently we are working to determine whether these genes share common regulatory elements. Further genetic and functional analyses of the differentially expressed genes along with possible common transcription factors that regulate these genes will provide us with a better understanding of the pathophysiological nature of otosclerosis.

Association of Angiotensinogen G-6A, C4072T, C6309T and A12775G polymorphisms with hypertension in Mexican population. *E. Balam-Ortiz[1], R. Gutierrez-Aguilar[1], J. Estevan-Baz[2], A. Esquivel-Villarreal[2], A. Elizalde[2], T. Gil[2], K. Carrillo[1], C. Rangel[1], V. Espinosa[1], L. Alfaro[1], A. Contreras[1], G. Jimenez-Sanchez[1]* 1) National Institute of Genomic Medicine, Mexico; 2) North Central Hospital, Mexico.

In Mexico, hypertension has a prevalence of 30.8% in population >20 years. It is a risk factor for heart and renal failure, myocardial infarction and cerebral vascular disease. Different genetic variants have been reported to be associated with hypertension, including those in angiotensinogen (*AGT*), adrenergic beta-1 receptor (*ADRB1*), angiotensin-II receptor type 1 (*AGTR1*). All these genes are involved in regulation of blood pressure. To analyze association of these variants with hypertension in Mexicans, we conducted a case-control study in 230 cases and 80 controls >65 years old from Mexico City. We genotyped 9 SNPs of *AGT*: C-532T(rs5046), G-218A(rs5049), A-20C(rs5050), G-6A(rs5051), C3889T (rs4762), C4072T (rs699), C6309T(rs2493132), A11535C(rs7079) and A12775G(rs943580); 2 SNPs of *ADRB1*: A145G (1801252), C1165G(1801253); and 1 SNP in *AGTR1*: A1166C(rs5186), using TaqMan assays (AB). We determined allele frequencies, Hardy-Weinberg equilibrium (HWE), and odds ratios (ORs) in the dominant, recessive, additive model, of each genetic variant. All variants were in HWE with a genotyping call rate higher than 95%. No association with hypertension was shown for polymorphisms in *ADRB1* and *AGTR1*. In contrast, four polymorphism in *AGT* showed a significant association with hypertension in: G-6A (OR 4.64; CI 95%=1.8-11.5; p=0.00008 in a recessive model); C4072C (OR 4.42; CI 95%=1.74-11.2; p=0.0009 in a recessive model); C6309T (OR, 12775A>G (OR 5.6; CI 95%=2.3-13.3; p=0.00004 in a recessive model); and A12775G (OR 5.2; CI 95%=1.9-13.8; p=0.00033 in a recessive model). We identified association of four variants of the *AGT* with hypertension in Mexican Mestizos. We are currently increasing the number of cases and controls in our study, and constructing haplotypes and analyze their association to hypertension in Mexicans. These studie will contribute to better understand the genetic basis of cardiovascular diseases in Mexicans.

Drosophila NnaD mutant flies model *Purkinje cell degeneration* mouse phenotypes and suggest a role for Nna proteins in mitochondrial function and mitochondrial dynamics. *R. Zahra[1], L. Chakrabarti[1], S. M. Jackson[1], L. J. Pallanck[2], A. R. La Spada[1]* 1) Depts of Laboratory Medicine; 2) Genome Sciences University of Washington, Seattle, WA.

The Purkinje cell degeneration (*pcd*) mouse is a recessive model of neurodegeneration, involving cerebellum and retina, with a phenotype of ataxia and blindness. Loss-of-function of the Nna1 gene causes *pcd*, and the Nna1 gene encodes a protein possessing a putative carboxypeptidase activity; however, neither the target substrates for Nna1 action nor the molecular pathways regulated by Nna1 are known. The fruit fly, *Drosophila melanogaster*, is a powerful system for modeling neurological phenotypes and defining mechanistic pathways for gene products of unknown function. As flies have a highly conserved orthologue of Nna1 (known as NnaD), we developed a fly model of *pcd* by characterizing a fly line carrying a P-element insertion at the NnaD gene. The NnaD fly model displayed shortened lifespan, and recapitulated key phenotypic features of the mouse Nna1 mutants including progressive retinal degeneration. Further studies of NnaD in *Drosophila* and Nna1 in *pcd* mice indicated that Nna proteins co-fractionate with mitochondria, and that loss of Nna function results in morphological and functional mitochondrial abnormalities. To investigate the role of NnaD in mitochondrial biology, we tested the hypothesis that Nna1 regulates mitochondrial dynamics and that defective mitochondrial dynamics result in oxidative stress sensitivity. NnaD mutant flies were crossed with flies carrying mutations in genes encoding the mitochondrial fission-promoting protein Drp1. Three independent Drp1 loss-of-function alleles ($drp1^{KG}$, $drp1^{T26}$ and *Df(2L)D20)* were all found to significantly suppress the NnaD phenotype. Furthermore, when we placed NnaD mutant flies on a diet containing Vitamin E and superoxide dismutase, we noted a significant extension in lifespan. These findings suggest that the Nna1 family of proteins play an important role in maintaining normal mitochondrial function through the regulation of mitochondrial dynamics, and that neurodegenerative phenotypes in *pcd* mice likely have a mitochondrial basis.

Designing Pools for High-Throughput Resequencing. *S. Prabhu, I. Peer* Department of Computer Science, Columbia University, New York, NY.

Resequencing genomic DNA from pools of individuals is an efficient strategy to detect new variants in targeted regions and compare them between cases and controls. However, naive designs of disjoint pools prevalent today obscure the individual identity of carriers of such variants. We present a framework for overlapping pool design, where each individual sample is resequenced in several pools. Upon discovering a variant, the set of pools where this variant is observed is the telltale of the identity of its carriers. We formalize the mathematical framework of such pool designs, and the requirements from such designs. We specifically address three practical concerns for pooled resequencing designs: (1) False positives due to errors introduced during amplification and sequencing; (2) False negatives due to undersampling particular alleles aggravated by non-uniform coverage; and consequently (3) Ambiguous identification of individual carriers in the presence of errors. We build on theory of error correcting codes to design pools that overcome these pitfalls. We show that in practical parameters of resequencing studies, our designs guarantee high probability of unambiguous carrier identification, while maintaining benchmarks of naive pools in terms of sensitivity, specificity and the ability to estimate allele frequencies.

Methylation profiling in Silver Russell Syndrome. *M. Penaherrera[1], S. Weindler[2], M. I. Van Allen[1], S. Langlois[1], W. Robinson[1]* 1) Dept Medical Genetics, Univ. British Columbia, Vancouver, BC, Canada; 2) Faculty of Medicine, University of Leipzig, Germany.

Silver Russell Syndrome (SRS) is a genetic disorder characterized by pre and postnatal growth deficiency, dysmorphic facial features, relative macrocephaly and body asymmetry. Efforts to determine the genetic basis of this condition have been compounded by its heterogenous nature, both at the clinical and molecular level. In previous studies of SRS, approximately 10% of cases presented with upd(7)mat and around 30% of the cases were associated with epigenetic mutations in 11p15. As part of an ongoing study of 22 SRS patients, two cases were identified as upd(7)mat and 8 cases (36%) showed hypomethylation for *IGF2/H19* ICR1 compared to age matched controls using a single nucleotide primer extension based assay (SNuPE). Clinical features were not significantly different in the ICR1-hypomethylated group. To identify additional alterations in methylation in SRS we used the Illumina Golden Gate Methylation Cancer Panel I, which assays 1505 CpG sites in a total of 807 genes, to test 21 SRS patients and 14 age matched controls. Sixty seven of these CpG sites are associated with 29 imprinted genes. Comparing the ICR1-hypomethylated group (N=8) with controls, the most significant methylation change was a reduced methylation at a CpG in the promoter region of *H19* ($p<0.001$). A positive correlation was found between the percent methylation from *H19*-ICR1 SNuPE and the average-beta methylation values from *H19*_P1411 Illumina, for the combined data of patients and controls ($r= 0.76$). Five additional CpG sites showed altered methylation (at $p<0.01$), though these did not include imprinted genes and this number was not more than expected by chance. This suggests that the changes in methylation at *H19* do not reflect global changes in methylation in this group. Two CpG sites (non-imprinted genes) were significant at the $p<0.01$ cut-off for the SRS group that showed normal methylation for ICR1. Several of the most interesting candidate changes, involving genes known to regulate growth, are being followed up by pyrosequencing.

Mecp2 Null Mice Display Multiple Skeletal Abnormalities. *R. D. O'Connor[1], M. Zayzafoon[2], M. C. Farach-Carson[1], N. C. Schanen[1,3]* 1) Department of Biological Sciences, University of Delaware, Newark, DE; 2) Department of Pathology, University of Alabama at Birmingham, Birmingham, AL; 3) Laboratory for Human Genetics, Nemours/A.I. duPont Hospital for Children, Wilmington, DE.

Rett Syndrome (RTT), a neurodevelopmental disorder, is most often caused by inactivating mutations in the X-linked gene encoding a regulator of epigenetic gene expression, methyl CpG binding protein, MeCP2. Clinical data show that, along with neurological defects, females with RTT frequently have marked decreases in Bone Mineral Density (BMD) beyond that expected from disuse atrophy. Our work with a Mecp2 null mouse model, Mecp2 -/yBIRD, reveals a difference between the wild-type and null mice, with the Mecp2 -/yBIRD mice having significantly shorter femurs and an overall reduced skeletal size. Histological studies have highlighted a shortened growth plate as well as decreased trabecular bone in the primary spongiosum of Mecp2 null femurs by 21 days of age, prior to the onset of neurological symptoms. Additionally, the trabeculae in the primary spongiosum of 60 day old Mecp2 -/yBIRD mice were abnormally shaped and hypercellularity was noted in the marrow space. Both histological and histomorphometrical analyses have shown reductions in the cortical bone parameters of Mecp2 null mice. It does not appear that these decreases in bone are owed to a primary effect on osteoclasts, as osteoclast numbers were comparable between wild-type and null animals. Also of note, serum calcium and phosphate levels were unchanged in Mecp2 -/yBIRD mice, consistent with that seen clinically in RTT patients. We speculate that Mecp2 deficiency leads to a primary dysregulation of genes critical for regulation of bone growth, differentiation and mineral homeostasis. Several genes essential to proper bone formation and maintenance of bone and calcium homeostasis have been identified as likely targets of Mecp2 in a differentiating osteoblast cell system. Current studies are underway to determine the functional significance of Mecp2 deficiency on candidate gene expression and function in osteoblastic cells.

The Autism Chromosome Rearrangement Database (ACRD): Annotation of Genomic Structural Changes in Autism Spectrum Disorder. *C. R. Marshall, J. Skaug, B. Kellam, D. Pinto, M. Manker, L. Feuk, A. Lionel, A. M. Joseph-George, S. W. Scherer* The Centre for Applied Genomics and Program in Genetics and Genome Biology, The Hospital for Sick Children and University of Toronto, Toronto, ON, Canada.

Large cytogenetically detectable chromosomal anomalies together with de novo submicroscopic copy number variants (CNVs) are associated with up to 10% of Autism Spectrum Disorder (ASD) cases. However, many studies lack the sample size to detect recurrent/overlapping loci and thus integration of multiple datasets is necessary to prioritize further screening of candidate loci. The Autism Chromosome Rearrangement Database (ACRD) is a comprehensive and curated catalogue of structural variation related to autism extracted from publicly available literature and unpublished data (http://projects.tcag.ca/autism/). The ACRD facilitates the interpretation of ASD structural variation data in relation to previously published work thus providing a valuable resource for both clinicians and researchers. The current content of the ACRD is based on results from 279 peer reviewed research articles (including case studies and whole genome scans) and unpublished data, representing a total of 887 entries. These structural variation entries are composed of 171 apparently balanced changes (including 48 inversions and 123 translocations), 323 large cytogenetically detectable unbalanced changes, and 52 CNVs (defined as gains and losses of DNA segments >1kb in size not detected by conventional karyotyping). Currently, the data are represented in table format (BioXRT) and in a genome browser format where molecular breakpoints are defined. The genome browser is based on the widely used GMOD software (GBrowse) and is ideal for viewing structural variation in relation to other genomic features, such as genes and segmental duplications. Moreover, selective data from ACRD is entered into the DECIPHER database. Here we present an overview of ACRD along with future plans for its expanded content, integration of karyotypic and CNV data, and enhanced presentation. We welcome submission of data and comments regarding the database from the research community.

Metabolic pathway transcriptional profiling reveals a signature of primary mitochondrial dysfunction that normalizes with antioxidant therapy. *M. J. Falk*[1], *Z. Zhang*[2], *D. L. Gasser*[3] 1) Human Genetics/Pediatrics, CHOP; 2) Biomedical Informatics, CHOP; 3) Dept of Genetics, UPENN, Philadelphia, PA.

Mitochondrial disease therapeutics is complicated by limited understanding of the cellular mechanisms mediating widely variable phenotypic findings. Applying metabolic pathway clustering to global genome transcriptional profiling presents a powerful opportunity to survey simultaneously very large datasets while reducing the complexity of describing metabolic regulation. We utilized this approach in two evolutionarily divergent animal models to identify cellular adaptations to single gene defects causing primary mitochondrial dysfunction. Results in *C. elegans* mutants for nuclear-encoded subunits of respiratory chain complexes I, II, and III (Falk et al, *Molecular Genetics and Metabolism*, 2008) are highly similar to those obtained in *M. musculus* mutants for Pdss2, a Coenzyme Q biosynthetic pathway enzyme (Peng et al, *PLoS Genetics*, 2008). Specifically, primary mitochondrial mutants across evolution concordantly upregulate 15 basic cellular metabolic pathways involving carbohydrate, amino acid, and fatty acid metabolism, as well as cellular defenses. Additional expression studies have now been undertaken in *Pdss2* mutant mice whose lethal kidney disease can be prevented with oral anti-oxidants to determine if metabolic pathway expression alterations normalize with therapy, and to specify which secondary metabolic alterations may be contributing to the disease phenotype. Probucol, an antioxidant/anti-hyperlipidemic agent, appears to normalize hepatic expression of pathways involved in carbohydrate, fatty acid, and steroid metabolism, P450 and other cellular defenses, and the PPAR signaling pathway in B6.*Alb/cre,Pdss2*$^{loxP/loxP}$ liver-conditional knockouts. Expression profiling in missense animals treated with Coenzyme Q or Probucol is in progress. Importantly, this work suggests identification of a systems biology-based 'biomarker' of primary mitochondrial dysfunction may be possible in humans. Furthermore, elucidation of common pathway alterations may permit the development of specific pathway-targeted therapies in the difficult to treat class of mitochondrial disorders.

Genetic variation of *CYP2D6* gene in the Mexican population. *A. Contreras, I. Silva-Zolezzi, T. Monge, L. Alfaro, S. Hernandez, H. Miranda, K. Carrillo, G. Jimenez-Sanchez* National Institute of Genomic Medicine, Mexico.

Genetic variation influence how humans respond to commonly used drugs. *CYP2D6* encodes a member of the cytochrome p450 superfamily, which are monooxygenases that catalyze many reactions involved in drug metabolism. This protein metabolizes about 20% of commonly prescribed drugs, such as metoprolol, an adrenergic-blocking drugs; paroxetine and fluoxetine, antidepressants; and tamoxifen and vinblastine, anticancer agents. This gene is highly polymorphic in the population and its enzymatic activity shows a high degree of interindividual and interethnic variability caused in part, by genetic variation. *CYP2D6* is located near two cytochrome P450 pseudogenes: *CYP2D7* and *CYP2D8*. Certain alleles result in a poor metabolizer phenotype. To characterize the genetic variation of *CYP2D6* in Mexicans, we resequenced the region corresponding to 9 exons and 9 introns in 96 samples of Mestizo population from two distant states of Mexico: Guerrero and Sonora. PCR products were subject to bi-directional sequencing (BigDye Terminator, Applied Biosystems). The resulting sequence fragments were analyzed using DNASTAR Lasergene (SeqMan Module). We observed 34 previously reported SNPs (14 in exons and 20 in introns), and identified 7 novel SNPs (3 in exons and 4 in introns) and 1 novel gene conversion *CYP2D6/2D7* in exon 2. This gene conversion was observed in Guerrero with a frequency of 4% and was not found in Sonora, so we hypothesize it is of local Amerindian origin. A more detailed study including 4 indigenous populations from Mexico is underway to confirm this hypothesis. This gene conversion may have a functional effect because its predicted change of amino acids is located near the proteins active site. To test this hypothesis, we are designing an *in vitro* study to analyze the effect of this mutation. Characterization of all different novel and known genetic variations in this Mexican sample is in progress. These results contribute to describe *CYP2D6* diversity in populations from different geographic and ethnic origins, and will help clarify the relationship between ethnicity and phenotype to improve implementation of pharmacogenomics in the clinical practice.

Genetic variation at chromosome 8q24 and risk of colon cancer. *C. Hutter[1], M. Slattery[2], D. Duggan[3], J. Muehling[3], K. Makar[1], L. Hsu[4], B. Caan[5], J. Potter[1], U. Peters[1]* 1) Cancer Prevention, FHCRC, Seattle, WA; 2) Internal Medicine, Univ. of Utah Health Sciences Center, Salt Lake City, UT; 3) Translational Genomics Research Institute, Phoenix, AZ; 4) Biostatistics, FHCRC, Seattle, WA; 5) Division of Research, Kaiser Permanente Medical Care Program, Oakland, CA.

First results from genome-wide scans and subsequent replication studies have shown that single nucleotide polymorphisms (SNPs) in chromosomal region 8q24 are associated with prostate, breast and colon cancer. Independent associations have been found in three regions of 8q24 for prostate cancer. In contrast, for colon cancer statistically significant associations have been restricted to a region between 128.47 and 128.54 Mb. This study investigated common genetic variants from this 71kb region and risk of colon cancer in a large population-based case-control study of 1594 colon cancer cases and 1935 controls. We genotyped 11 SNPs and used logistic regression to calculate odds ratios (ORs) and 95% confidence intervals (95% CIs) based on a log additive model. We examined gene-environment interactions for known colon cancer risk factors (obesity, physical activity, smoking, non-steroidal anti-inflammatory drug use, family history of colon cancer and sex). All analyses were adjusted for age, sex and study center. Three SNPs were statistically significantly associated with risk of colon cancer. The strongest association was for rs9297756 (OR for the A allele under a log additive model: 1.22; 95% CI, 1.07-1.39; p=0.002). The rs6983267 and rs10505477 SNPs are in high LD (r2=0.93), and each showed a significant association with colon cancer (OR for the G allele at rs6983267 or T allele at rs10505477: 1.12; 95% CI, 1.01-1.23; p=0.02). Similar results were obtained when the analysis was restricted to whites (1461 cases; 1813 controls). The associations did not differ for proximal vs. distal anatomical location, and no statistically significant gene-environment interactions were observed for the factors examined. In conclusion, this study replicates the association between SNPs at 8q24 and colon cancer risk. Further research is needed to elucidate the biological mechanism underlying this association.

Pedigree data analysis using RELTEST, GRR, and MERLIN. *K. M. Lewis[1], S. G. Buxbaum[2], L. Ekunwe[2]* 1) Jackson Heart Study, Tougaloo College, Jackson, MS; 2) Jackson Heart Study, Jackson State University, Jackson, MS.

Abstract: Genetic information from individuals who were among the family study component of the Jackson Heart Study was used for this analysis. This data comprised 374 autosomal microsatellite markers typed by the Marshfield Mammalian Genotyping Center. Two programs, GRR (Graphical Relationship Representation) and RELTEST (a program in the S.A.G.E. package), were used to test the relatedness between relative pairs. This was done in order to decrease the amount of error within the family pedigree structures. Because the participants are all from the same metropolitan area, it seemed reasonable to expect to find relatedness across pedigrees, as well as within them. This was tested, and where clear cases of relatedness were found using microsatellite markers, pedigrees were merged. In some cases, we were able to identify unknown individuals and link pedigrees to one another. Where some links were still unclear, for example, in cousin pairs, we made use of markers on the sex chromosomes (Y only and/or X), and estimated haplotypes using MERLIN. Through the use of these programs, we have been able to minimize the number of putative unrelated individuals who are actually close relatives. We began with 291 families and found that these relationships are better described by 264 pedigree structures. This work was done to increase the power of future genetic linkage and association analyses, whether using microsatellite or single nucleotide polymorphism data, and to help to minimize false positive results in such analyses. This research from the Jackson Heart Study is supported by NIH contracts N01-HC-95170, N01-HC-95171, and N01-HC-95172 provided by the National Heart, Lung and Blood Institute and the National Center for Minority Health and Health Disparities.

Forms of Provision of Genetic Testing Services in Japan. *M. Watanabe[1], K. Dobashi[3], A. Tsuchiya[2], T. Oohata[2], T. Sumida[2], K. Muto[1], Y. Nakamura[1], F. Takada[2]* 1) Dept. of Public Policy, Inst. of Med Sci, Univ.of Tokyo, Tokyo, Japan; 2) Dept. of Clinical Genetics, Grad. School of Med.Sci. Kitasato Univ, Kanagawa, Japan; 3) Japan Bioindustry Association.

[Aim]Genetic testing is now provided in various venues outside of the specialized sites for clinical genetics, represented by direct-to-consumer marketing. Issues of DTC genetic testing services differ according to the way they are provided. Thus in order to promote detailed discussion on the genetic testing services provided outside of classic clinical genetics, it is necessary to classify the current forms of provision. In this presentation, varieties of ways in which genetic testing services are provided in Japan are introduced, and related agendas are discussed. [Method] Questionnaire surveys to 45 companies related to DTC genetic testing services and 97 general clinics providing multi factorial conditions were conducted in Japan between May and October 2007. [Result] 11 companies and 22 clinics were responded. [Discussion] Ways of marketing genetic testing services outside of classic clinical genetics in Japan can be classified in 7 styles. The biggest market is being created in the form that uses private general clinics as distributors. The survey to the clinics revealed that the most common reason for providing lifestyle test at the clinics is promotion of motivation for medical intervention. It arouses the question whether this reason of providing genetic test yet clinically validated is permissible or not. In Japan, the answer to this question has yet been sought for.

Comprehensive analysis of causative genes in sporadic ALS patients reveals multiple rare variants potentially associated with disease risks. *Y. Takahashi, H. Ishiura, J. Goto, S. Tsuji* Dept Neurology, Univ Tokyo, Tokyo, Japan.

[Objectives] To identify novel rare variants associated with disease risks in sporadic ALS (SALS) patients. [Background] Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disorder characterized by progressive degeneration of upper and motor neurons. Familial ALS (FALS) comprises 5 - 10 percent of the cases, whereas the remaining patients are sporadic. Current approaches to identify genetic risks of SALS basically employ genome-wide association studies based on common disease-common variant hypothesis, whereas the resequencing of disease-related genes is emerging as an intriguing alternative approach to identify rare variants associated with disease risks. [Materials and Methods] DNA samples obtained from 120 sporadic ALS patients with informed consent, who fulfilled the revised El Escorial and Airlie House diagnostic criteria, and 250 controls, were used in this study. We have developed a DNA microarray-based resequencing system to facilitate the comprehensive analysis of causative genes of FALS. Entire exons and flanking introns of 7 genes, *SOD1*, *ALS2*, *DCTN1*, *VAPB*, *ANG*, *CHMP2B* and *TARDBP*, were amplified with specific primers and hybridized to the custom-made DNA resequencing microarrays, TKYALS01 and TKYALS02. The rare variants identified in the above strategy were further analyzed in the controls. [Results] In total, 30 variants in addition to 2 novel *SOD1* mutations and 1 known *SOD1* mutation were found. Nineteen of the 30 variants (63 percent) were novel, including 3 nonsynonymous variants in *DCTN1*, *ANG* and *TARDBP*. These 3 variants were not found in 250 controls (500 chromosomes), and highly conserved among species. [Conclusion] Comprehensive analysis of causative genes in sporadic cases revealed not only causative mutations but also novel nonsynonymous variants, raising the possibility that substantial proportion of SALS patients may be accounted for by these rare variants. Large scale genetic studies as well as functional analysis of these extremely rare variants were necessary to further investigate the contribution to genetic risks and pathogenesis of SALS.

Bootstrap- vs. Likelihood-based methods to reduce selection bias: variance, confidence interval estimation and computational feasibility in large-scale genetic mapping studies. *L. L. Faye[1,2], S. B. Bull[1,2], L. Sun[1,3]* 1) Public Health Sciences, University of Toronto, Toronto, ON, Canada; 2) Samuel Lunenfeld Research Institue, Mount Sinai Hosplital, Toronto ON, Canada; 3) Hospital for Sick Children, Toronto ON, Canada.

Selection bias in genetic effect parameters (e.g. heritability, odds ratio) is prevalent in genome-wide mapping studies because the same sample is typically used for both gene discovery and effect estimation (e.g. linkage analysis - Goring et al, 2001; case-control association - Garner, 2007). Effect estimators that reduce selection bias have recently received much attention and mainly fall into two categories: bootstrap resampling-based methods (Sun and Bull, 2005; Wu et al, 2006; Yu et al, 2007), and likelihood-based approaches (Zollner and Pritchard, 2007; Zhong and Prentice, 2008; Ghosh et al, 2008). The statistical resampling approach is flexible and can be readily extended to detection of multiple linked markers but is computationally expensive. In contrast, the maximum-likelihood-based approach directly models the power to detect linkage or association but considers each marker in isolation. To quantify the bias, relative variance, and confidence interval (CI) coverage of the two approaches, we simulated single-marker datasets and tested for genetic association under the assumption of a normally distributed parameter estimate. We developed a two-level bootstrap method to obtain CIs for the resampling-based effect estimates. In scenarios with low power and stringent testing criterion, both estimators improve estimation accuracy, but a moderate amount of bias remains. On average the MLE over-corrects while the bootstrap estimator under-corrects. Comparison of the bootstrap-based CI with the likelihood-based CI method found that both methods yield CIs with slightly higher than nominal coverage, but the bootstrap 95%; CI is, on average, shorter than the likelihood 95%; CI. We also demonstrate the feasibility of the resampling-based methods through application to existing high-throughput GW association studies.

Proteomic analysis of astrocytoma cell lines stimulated with progesterone. *M. Rodriguez-Dorantes[1], L. Castellanos-Tapia[1], K. G. Calderon-Gonzalez[1], E. Cabrera-Muñoz[2], I. Camacho-Arroyo[2], J. P. Reyes-Grajeda[1], J. L. Gallegos-Perez[1], G. Jimenez-Sanchez[1]* 1) National Institute of Genomic Medicine, Mexico; 2) School of Chemistry, UNAM, Mexico.

Brain tumors produce several neurological symptoms as a result of their size, localization and invasive qualities. They represent 10-15% of all human neoplasias. Progesterone (P4) has been associated with tumoral growth. To determine the effects of progesterone (P4) and its antagonist, RU486, on protein expression profiles we used two human astrocytoma cell lines with different evolution grade (U373, grade III; and D54, grade IV). We treated each cell line with either vehicle, P4(10nM), RU486(10M) or P4+RU48. After 48 hrs, cells were collected, homogenized and proteins were extracted and quantified. 2D gel electrophoresis of total proteins was performed. Gels were Coomassie blue-stained and analyzed using Image Master 2D. Differentially expressed spots were extracted, trypsin-digested and desalted, prior to mass spectrometry analysis. Proteins were identified using the Paragon algorithm. Comparative analysis with P4 identified increased presence of Relaxin 3 receptor 2, glucosidase 2 subunit beta precursor, heat shock 70KDa, phosphoglycerate kinase 1, keratin type II cytoskeletal 2, stress 70 protein mitochondrial precursor, superoxide dismutase mitochondrial precursor, alpha crystallin B chain, triosephosphate isomerase, ubiquinol-cytochrome c reductasa, trypsin 3, precursor translationally-controlled tumor protein, and peroxiredoxin-6 glucosidase. Stimulation with RU486 showed increased expression of vimentin, lamin B2, and phosphatidylethamine-binding protein 1. Some of these proteins are related with tumor growth, aggressive phenotype or as a marker in tumors. These differentially expressed proteins in U373 and D54 cell lines are associated with several cellular processes such as cytoskeleton organization, biotransformation, and signal transduction. Our results indicate that P4 and its antagonist RU486 exert different effects in the protein profile of astrocytomas, suggesting a potential role of progesterone in these tumors.

Does MDS with der (1;7) constitute a distinct risk group? A retrospective analysis of clinical/pathological features compared to -7/del(7q) MDS. *M. L. Slovak[1], M. O'Donnell[2], D. Smith[3], K. Gaal[4]* 1) Dept Cytogenetics, City of Hope, Duarte, CA; 2) Division of Hematology and Hematopoietic Cell Transplantation; 3) Division of Information Sciences; 4) Dept of Anatomic Pathology.

The der(1;7)(q10;p10) has been reported in ~1-3% of the myelodysplastic syndromes (MDS) and less commonly in acute myeloid leukemia (AML) and the myeloproliferative disorders. In MDS, the der(1;7) is considered a variant of the del(7q)/-7 subgroup and assigned a POOR risk karyotype score in the IPSS, a clinical scoring system that estimates overall survival (OS) and risk of AML transformation. A recent report (Leukemia 21:992-7, 2007) suggests der(1;7) MDS should be considered a discrete MDS subgroup with an intermediate karyotype score. Moreover, the der(1;7) reported MDS cases are predominantly Asian. In this study, we performed a retrospective study to define the clinical-pathological features of 13 MDS patients (pts) with der(1;7) evaluated at the City of Hope and compared their features with 54 MDS pts with either del(7q) (n=11) or -7 (n=44). When compared to the del(7q)/-7 MDS cases, the der(1;7) pts showed a male predominance, an older age at diagnosis (mean = 13 yrs older, p = 0.0168), low platelet counts (p=0.0026), less trilineage dysplasia, fewer secondary aberrations, and lower blast counts. We found that der(1;7) patients did not differ compared to the del(7q)/-7 MDS pts with respect to the subtypes of MDS either by the WHO (p = 0.562) or FAB (p = 0.637) classifications. Increased eosinophilia and higher hemoglobin levels, as reported previously, were not observed in the current study. The proportion of therapy-related MDS or t-MDS was 25% for der(1;7), 37% for -7 and 55% for del(7q)(p=0.434). Five year OS did not differ among the three MDS groups [der(1;7) = 44.4% vs del(7q) = 35.4% vs -7 = 22.4%), p=0.69] with ~50% of each cohort receiving a stem cell transplant. Based on these preliminary data, der(1;7) MDS is associated with some unique clinical features but reassignment of der(1;7) MDS from poor to intermediate appears to be premature. A large scale international study to further characterize this unique MDS subgroup is suggested.

Variation in the Nicotinic Cholinergic Receptor Gene Cluster *CHRNA5-CHRNA3-CHRNB4* and Its Interaction with Substance Dependence Influence Cognitive Flexibility. *H. Zhang[1,2], H. R. Kranzler[3], J. Poling[1,2], J. Gelernter[1,2]* 1) Dept of Psychiatry, Yale University School of Medicine, New Haven, CT; 2) VA Connecticut Healthcare System, West Haven, CT; 3) Dept of Psychiatry, University of Connecticut School of Medicine, Farmington, CT.

Objective: We investigated whether variation in the nicotinic receptor gene cluster *CHRNA5-CHRNA3-CHRNB4* and its interaction with substance dependence (SD) can affect cognitive flexibility. Method: Working memory of 544 African Americans (AAs) (468 cases with SD and 76 controls) and 445 European Americans (EAs) (314 cases with SD and 131 controls) were assessed using the Wisconsin Card Sorting Test (WCST). Influence of markers in this gene cluster and marker-SD interaction on cognitive flexibility was analyzed. Effects of haplotypes and haplotype-SD interaction on cognitive flexibility were also examined. Results: *CHRNB4* rs11637890 minor allele was associated with an improved performance on three WCST domains (non-perseverative errors: P=0.015; conceptual responses: P=0.040; and categories completed: P=0.010) in AA controls (but not in AA cases). A significant interactive effect of this marker and SD on these three domains was also observed. Haplotype CTCTGCG was shown to have a beneficial effect on performance in at least two WCST domains in AA controls (non-perseverative errors: P=0.0166; conceptual responses: P=0.022). An interactive effect of haplotype CTCTGCG and SD on performance in these two domains was also evidenced. In EAs, Two *CHRNA5* SNPs (rs684513 and rs615470) and two *CHRNA3* SNPs (rs6495307 and rs2869546) (these markers being in high linkage disequilibrium) were associated with cognitive flexibility on at least two WCST domains (perseverative responses: P=0.013-0.035 and perseverative errors: P=0.009-0.033) in controls (but not in cases). A significant interactive effect of these four markers and SD was observed in the same two WCST domains (perseverative responses: P=0.032-0.050 and perseverative errors: P=0.020-0.050). Conclusions: Variation in the gene cluster *CHRNA5-CHRNA3-CHRNB4* appears to affect cognitive flexibility differentially in AAs and EAs, and SD traits could moderate this effect.

Association of TPO SNPs with type 1 Diabetes in North-west Colombia. *J. Gutierrez-Achury[1], J.-M. Alfaro[1], V. Balthazar[1], G. Bedoya[2], F. Uribe-Londono[3], A. Ruiz-Linares[2,4], N. Pineda-Trujillo[1,5]* 1) Mapeo Genético, Departamento de Pediatría y Puericultura, Facultad de Medicina, Universidad de Antioquia, Medellín-Colombia; 2) GENMOL, Universidad de Antioquia, Medellín-Colombia; 3) Endocrinologia y Metabolismo, Facultad de Medicina, Universidad de Antioquia, Medellín-Colombia; 4) Department of Biology, University of London, UK; 5) Pediaciencias, Departamento de Pediatría y Puericultura, Facultad de Medicina, Universidad de Antioquia, Medellín-Colombia.

Type 1 diabetes (T1D) is a complex trait in which genetic and environmental factors are involved. We have previously found linkage and association of T1D to 2p25. Our purpose was to analyze the role of the gene TPO in the susceptibility to T1D in Colombian families. One hundred familial trios with T1D from Antioquia-Colombia were analyzed. In patients sera autoantibodies against anti-GAD, anti-IA2 and anti-TPO were tested. Two SPNs intragenic to TPO were tested (rs4927611, rs732609). These two markers were chosen considering that the polymorphism changes the encoded aminoacid and that the MAF<=0.3. The location for the SNPs was determined by using the dbSNP database at NCBI. SNPs typing was done by PCR-RFLP and Tetraprimer-ARMS methods. Genetic association analysis was done by the transmission desequillibrium test (TDT). Positive autoantibodies were found in 73%, 41% and 17% for anti-IA2, anti-GAD and anti-TPO, respectively. Together, 86% of the affected individuals were positive for at least one auto-antibody. SNP rs4927611 showed a P 0.0561 and for SNP rs732609 a P0.05 was obtained. For the haplotype analysis a P 0.002 was found. Autoimmunity was found in similar conditios to previous reports. It was also found that both SNPs at TPO gene are associated to the disease. Furthermore, a haplotype characterized by alleles at both SNPs was associated with increased risk to T1D. Our results suggest a causative participation of the gene TPO in T1D. Replication in an independent sample is required in order to confirm this finding. Acknowledgement: This study was supported by Colciencias grant # 111534319156 and CODI-Universidad de Antioquia grant # 8704-2449.

Gene Expression in human samples using SOLiD Next Generation Sequencing. *C. Barbacioru[1], M. Barker[1], J. Gu[2], S. Kuersten[2], D. Wang[1], R. Nutter[1], R. Wicki[1], R. Setterquist[2], F. De La Vega[1], G. Spier[1]* 1) Applied Biosystems, Foster City, CA; 2) Ambion Inc - An Applied Biosystems Business, Austin, TX, USA.

Analysis of gene expression patterns provides valuable insight into the role of differential expression in biological and disease processes. High density microarrays -- the standard for global gene expression analysis -- are limited in their dynamic range and can be ineffective at measuring genes expressed at a low level. Additionally, hybridization based platforms require an a priori knowledge of the mRNA sequences and are therefore unsuited for hypothesis free RNA discovery type of studies. The SOLiD System overcomes the limitations of microarray technologies by providing an ultra high throughput sequence-based platform for quantitative measurement of expression of RNA molecules. SOLiD produces 100s of millions of short reads (35-50bp) in a single run, requiring low sample input. This allows us to explore gene expression profiles at the whole genome scale, without prior RNA sequence knowledge enabling an entirely new scale of biological experimentation (alternative splice forms, allele specific expression, non-coding RNA etc.), with large dynamic range, a tunable depth of coverage for rare transcript discovery and quantification. Additionally, in this study we compare the results produced by this system with TaqMan measurements obtained from a large number of genes, generated in the original MAQC study.

Measurement of the downstream revenue generated by a clinical cancer genetics program. *K. M. Durda[2], W. S. Rubinstein[1,2], S. Weissman[1,2], S. M. O'Neill[1,2]* 1) Evanston NW Healthcare, Evanston, IL; 2) Northwestern Univ. Feinberg School of Medicine, Chicago, IL.

Background Clinical genetics centers are generally not financially self-sufficient based on directly billed services. However, these centers may generate indirect downstream revenue (DSR) for billable services that are performed in that hospital system as a direct result of medical recommendations stemming from a genetics evaluation. **Purpose** This study assessed the revenue indirectly generated from a clinical cancer genetics program, the Center for Medical Genetics (CMG), through its associated hospital system, Evanston Northwestern Healthcare (ENH). We wanted to determine if the CMG was generating DSR for ENH, and if so the amount of DSR. **Methods** We assessed total billing generated in a 4.5 year period by CMG patients who were carriers of cancer predisposing mutations. A chart review of patients with Hereditary Breast and Ovarian Cancer (HBOC) syndrome, Hereditary Non-Polyposis Colorectal Cancer syndrome (HNPCC), or Familial Adenomatous Polyposis syndrome (FAP)/MYH-associated polyposis (MAP) was done to determine whether billed encounters were attributable to the CMG; 19,117 lines of billing data were reviewed. **Results** Seventy-five of the 123 patients (61%) with available billing information had encounters at ENH that were attributable to the CMG. The DSR billed for these 75 patients was $1,708,956; this is $7,555 billed per person-year. Based on contractual deductions, actual DSR would be about half that amount. **Conclusions** This study found that the CMG generates a significant amount of DSR for ENH through increased cancer surveillance and prophylactic surgeries of patients with HBOC, HNPCC, and FAP. The DSR generated by mutation carriers with attributable billing encounters is substantially higher than the professional fees that are directly billed by the CMG for those patients. For each $1 billed directly by the CMG for the 75 patients with attributable encounters, ENH billed $17 in DSR. These data may be of interest to hospital administrators when making funding allocation decisions for genetics centers that are not financially self-sufficient based on direct revenues.

Sex-biased demographic processes shape genomic patterns of human diversity. *J. Wall[1], M. F. Hammer[2,3], F. L. Mendez[3], M. P. Cox[2], A. Woerner[2]* 1) Inst Human Genetics, Univ California, San Francisco, San Francisco, CA; 2) ARL Division of Biotechnology, University of Arizona, Tucson, AZ; 3) Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ.

We compared levels of genetic variability on the autosomes and the X chromosome from over 400 Kb of resequencing data from 90 different intergenic regions in a panel of 90 humans from six geographically diverse populations. After correcting for differences in mutation rates across chromosomes, we find that the relative levels of genetic variation are higher than expected on the X chromosome in all six human populations. We test a number of alternative hypotheses to explain the excess polymorphism on the X chromosome, including models of background selection, changes in population size, and sex-specific migration in a structured population. While each of these processes may have a small effect on the relative ratio of X-linked to autosomal diversity, our results point to a systematic difference between the sexes in the variance in reproductive success; namely, the widespread effects of polygyny in human populations.

Bilateral Aplasia of the Cochlear Nerves and Olfactory Bulb Agenesis in association with a SOX10 mutation. *C. P. Barnett[1], W. Reardon[1], S. Blaser[2], J. Gillis[1], L. Dupuis[1], A. V. Levin[3], P. W. Chiang[4], E. Spector[4], R. Mendoza-Londono[1]* 1) Clinical and Metabol. Genetics, Hospital for Sick Children, Toronto, Ontario, Canada; 2) Neuroradiology and Otolaryngology, Hospital for Sick Children, Toronto, Ontario, Canada,; 3) Ophthalmology and Vision Sciences, Hospital for Sick Children, Toronto, Ontario, Canada; 4) University of Colorado Health Sciences Centre, Denver, Colorado.

SOX10 is a transcription factor essential for neural crest cell migration and differentiation. Mutations in SOX 10 have been shown to cause autosomal dominant cases of Waardenburg-Shah syndrome (WS4, OMIM 148820), characterized by the combination of hypopigmentation and sensorineural hearing loss (SNHL) in association with Hirschsprungs disease. We report the first case of aplasia of the cochlear nerve due to a novel SOX10 mutation in a child with profound SNHL, severe visual impairment, central nervous system hypomyelination and cutaneous hypo-and hyperpigmentation. On examination at 17 months of age the patient was noted to have fair skin and hair and multiple areas of cutaneous hyperpigmentation including numerous café au lait spots and lentigines. Neuroimaging identified central nervous system hypomyelination, bilateral cochlear nerve aplasia, olfactory bulb aplasia and optic nerve hypoplasia. Initial investigations including karyotype, array comparative genomic hybridization (aCGH) and a full metabolic screen were normal. SOX 10 mutation testing was undertaken because of his constellation of clinical findings and a novel missense mutation of the highly conserved high mobility group (HMG) domain of SOX10 was identified (Q174P:c.521A>C). This patients findings are not typical of WS4 and suggest that awareness of these clinical features as a function of SOX10 mutation may result in further cases of non-classical phenotypes being diagnosed.

Implementation of an Oral Cleft Registry in the Philippines. *E. M. C. Cutiongco[1,2], C. D. Padilla[1,2], A. L. D. Sur[1] for the Philippine Oral Cleft Study Group* 1) Institute of Human Genetics, National Institutes of Health Philippines, Manila, Philippines; 2) Department of Pediatrics, College of Medicine-Philippine General Hospital, University of the Philippines Manila.

Oral clefts are among the most common congenital anomalies. Various literature estimate global incidence of oral clefts at 1 every 500-1000 livebirths, which varies with population and gender. Orientals, in particular, are at a higher risk for oral clefts. Numerous researches have expounded on the etiologies, treatment and prevention. The most relevant etiologic factors identified include maternal exposure to tobacco smoke, alcohol, micronutrient insufficiency, corticosteroids, exogenous estrogen, organic pollutants, and occupational chemicals. A surveillance system in the Philippines was established under the Philippine Oral Cleft Research Study Group composed of the Institute of Human Genetics, the Philippine Association of Plastic, Reconstructive and Aesthetic Surgeons Inc. (PAPRAS), the Philippine Society of Otorhinolaryngology - Head and Neck Surgery (PSO-HNS), Operation Smile Philippines Foundation Inc. (OSPF), and the Philippine Band of Mercy (PBM) in 2004. The Philippine Oral Cleft Registry aims to systematically collect, analyze, and interpret data on oral clefts including maternal history and demographic profile. There were a total of 1,838 cases reported. With an estimate of 1,600, 000 livebirths per year, the prevalence of reported cases is 0.51 per 1000 livebirths, which was lower than previously reported prevalence rates. There is a consistently observed male predominance in all of the reported cases with a male to female ratio of 1:1.5. Cleft of the hard and soft palate with bilateral cleft lip is the most common type observed. The Philippine Oral Cleft Registry is one of the first initiatives to implement a registry program for patients with oral clefts in the Philippines through a network of organizations and institutions. Continued surveillance through such registry will allow evaluation of existing programs and the promotion of new public health projects that will adequately deal with the preventable causes of oral clefts in the country.

Genetic predisposition to lung cancer and the association between cancer genetic susceptibility and long-term cigarette smoking. *S. Hwang[1], D. Levy[1], C. L. Rosenberg[2], L. Atwood[3], B. E. Kerger[2], J. M. Murabito[2]* 1) The National Heart, Lung, and Blood Institute's Framingham Heart Study, Framingham, MA; 2) Department of Medicine, Boston University School of Medicine, Boston, MA; 3) Department of Biostatistics, Boston University School of Public Health, Boston, MA.

Genome-wide-association-studies (GWAS) have demonstrated a significant association of SNPs on 15q24/15q25.1 with lung cancer. The characterized genes in this region include nicotinic acetylcholine receptor subunit genes, which are promising candidates for tobacco addiction. In the Framingham Heart Study (FHS), systematic medical records review enabled the classification of malignancies and the adjudication of coronary heart disease endpoints in study participants. Genotyping was conducted for 550, 000 SNPs in 9000 FHS participants across three generations. We examined the association of 21 SNPs in the 15p25 region with 90 lung cancer cases and 1521 cancer-free controls who has no family history of cancer. Three control groups: A) participants surviving to age 65 without family history of cancer, B) non-smokers in group A , and C) non-smoker who were free of coronary heart disease in group B. We applied the same association test for all cancer cases (n=860) and type-specific (breast n=184, colorectal n=108, prostate n=190) cancers. we tested for genetic association by applying survival analysis used a frailty model to adjust for familial correlation. Results of the analysis showed 2 SNPs with p-value less than 0.02 when comparing lung cancer cases with group C controls. The p-values for the same SNPs were less than 0.03 when compared with group B controls. Results of the study showed a consistently lower p-value with no-CVD controls for lung cancer. However, the lower statistical power is the main concern of this approach that requires further study.

PRDM8 is required for the terminal differentiation and maintenance of rod & cone bipolar cells in the mammalian retina. *D. Atan[1], C. Jung[1], K. Allen[1], M. Klein[2], D. Birch[2], R. R. McInnes[1]* 1) Hospital for Sick Children & University of Toronto, Canada; 2) Retina Foundation of the Southwest, Dallas, TX.

Knowledge of the transcriptional regulation of neuronal development has major implications for the understanding and therapy of disease. The transcription factor (TF) PRDM8 is a highly conserved Zn-finger protein of the PR domain family. We found that *Prdm8* is widely expressed in the developing CNS. Expression is maintained in adult retina, where the protein is abundant in bipolar cells, the major interneurons of the inner nuclear layer (INL) that relay visual signals to the optic nerve. In *Prdm8*$^{-/-}$ retinas at postnatal day 6 (P6), total bipolar (Chx10+) cell number & retinal morphology are normal, indicating normal bipolar cell specification. However, rod bipolar cell differentiation is impaired, since the rod bipolar marker, PKC, is undetectable at P6 and only weakly detected at P10. By adulthood, rod & cone Type 2 OFF bipolar cells are virtually absent, contributing to a 33% reduction in INL cell number. Consistent with the absence of rod bipolar cells, *Prdm8*$^{-/-}$ mice have b-wave deficits on dark-adapted ERG, confirming the compromised rod bipolar circuitry. To begin to identify Prdm8 target genes in bipolar cell development, we compared gene expression of *wt* vs *Prdm8*$^{-/-}$ retinas at P6 (FDR 10%), when normal numbers of mutant bipolar cells are present but poorly differentiated. Using the GoMiner gene ontology program, we found that genes involved in neurogenesis, neurite development and DNA-binding were over-represented in the *Prdm8*$^{-/-}$ retina ($p<0.05$). For example, *Vsx1* (a major OFF cone bipolar TF) is 80% down-regulated (by qPCR), and *Sox6* (a TF regulator of oligodendrocyte differentiation) & *Lhx4* (a bipolar cell-expressed TF) are 240% and 60% up-regulated, respectively. We conclude that i) Prdm8 is critical for the late differentiation and maintenance of rod and cone Type 2 OFF bipolar cells in the retina; ii) the differentially expressed TF genes of the P6 *Prdm8*$^{-/-}$ retina are strong candidates to be controlled by Prdm8, suggesting they may regulate late steps of bipolar cell differentiation; and iii) PRDM8 is a candidate gene, at 4q21, for human congenital blindness.

Differential expression signature in Stem Cells of non-syndromic cleft lip/cleft palate patients: A new approach to understand this orofacial malformation? *D. F. Bueno[1,2], G. Kobayashi[1], D. Yumi[1], C. Amaral[2], I. Zambra[1], M. Aguena[1], I. Kerkis[3], M. R. Passos-Bueno[1]* 1) Instituto de Biociencias, USP, Sao Paulo, Brazil; 2) SOBRAPAR, Campinas, Brazil; 3) Instituto Butantan, São Paulo, Brazil.

Non-syndromic cleft lip/palate (NSCL/P) is a complex disease determined by several interacting loci that are influenced by environmental covariates. In the present work we ivestigated if the genetic expression profile of affected NSCL/P differs from control dental pulp stem cells(DPSC). DPSC and adult stem cells extracted from other tissues are a reliable source of cells that can provide a closer parallel in understanding the evolution of NSCL/P embryonic development, given the fact that obtaining RNA from NSCL/P human embryos during embryonic development of lip and palate is unfeasible. We established DPSC cultures according to previous protocol (Costa et al, 2008). Total RNA was isolated from 5 control and 6 NSCL/P DPSC cultures. Gene expression experiments were performed using Human Gene 1.0 ST Array (Affymetrix) according to manufacturers protocols. Data was normalized using the RMA method from the Bioconductor Affy package. Two different methods were employed to select differentially expressed genes(DEGs):the Student's T-test using the MeV program and the Bioconductor Limma package. A total of 36 differentially expressed genes were identified using the Student's T-test with a $p = 0.01$ while 50 DEGs with best scores (B-value) were selected with Limma analysis. We verified 18 overlapping DEGs between the used methods. These 18 DEGs were clustered using the K-means and SOM (Self-organizing map) algorithms, available in Expander program. The highly homogenous clusters corroborated the differences between control and affected levels of expression. The DEGs were annotated with the Affymetrix NetAffx tool. Our preliminary data thus support a difference between the global signaling pathways of NSCL/P and control individuals. These results, once confirmed with different methodologies, will open new possibilities to study and understand the pathophysiology of NS CLP. CEPID/FAPESP, CNPq.

17q21.31 deletion syndrome: Are autoimmune disorders part of the phenotype? *S. Halbach, S. Schwartz, D. Waggoner* Department of Human Genetics, University of Chicago, Chicago, IL.

We present a 14yo girl with the findings of ASD, hydrocephalus, hypotonia, history of seizures, exotropia and amblyopia, sensorineural hearing loss, scoliosis, spondylolisthesis, eczema, chronic neutropenia, autoimmune primary adrenal insufficiency (confirmed by positive adrenal antibodies and 21-OH antibodies), vitiligo, alopecia, and autoimmune thyroid disease. Additionally, dysmorphic facial features including downslanting palpebral fissures, tubular pear-shaped nose, widely spaced teeth, high arched palate, and low-set ears were noted on exam. An extensive genetic work-up had been done previously, including a normal 46,XX karyotype and normal commercial, targeted microarray. Research-based whole genome SNP array revealed a 17q21.31 deletion of approximately 550 kb which was confirmed by FISH. This is similar in size to the previously defined critical region. Parental FISH studies were negative. More than 10 individuals with microdeletions of 17q21.31 have been described to date, ranging in age from 3 to 26 years, though none have been reported to have autoimmune findings. Although autoimmune disease could be an incidental finding in our patient, adrenal insufficiency is difficult to diagnose, potentially fatal, and can be treated; therefore, we feel that clinicians should be aware of this potential feature in 17q21.31 deletion syndrome. Included in the critical region is the CRHR1 (corticotrophin-releasing hormone receptor 1) gene which binds to corticotrophin-releasing hormone, a mediator of endocrine, autonomic, behavioral, and immune responses to stress. In mice lacking Crhr1, atrophy of the medulla of the adrenal gland and reduction of stress-induced release of ACTH and corticosterone have been observed. Conversely, overexpression of CRHR1 has been detected in Cushing disease. CRHR1 could be a candidate for concomitant autoimmune disease in 17q21.31 deletion syndrome.

A novel locus for autosomal dominant hereditary spastic paraplegia maps to chromosome 3q24-q26. *Y. Gong, P. Lin, Q. Liu, G. Gao, C. Shao* Institute of Medical Genetics, Shandong University School of Medicine, Jinan, Shandong 250012, China.

Hereditary spastic paraplegias (HSPs) are a group of neurodegenerative disorders characterized by progressive spasticity of the lower limbs. Clinically, HSPs are classified as either the uncomplicated or complicated form depending on whether spasticity occurs in isolation (uncomplicated HSP) or is associated with additional symptoms(complicated HSP). Moreover, the disorders manifest extremely genetic heterogeneity and considerable inter- and intra-familial variation in age at onset and severity of spasticity. To date, 38 HSP loci and 19 spastic paraplegia genes (*SPG*) have been identified. In this study, we evaluated a large Chinese kindred with autosomal dominant uncomplicated HSP. Forty individuals (18 affected, 22 unaffected) were included in this study. The study was approved by the ethical Committee in Shandong University School of Medicine and informed consent was obtained from all participants. All patients had typical signs of spastic paraplegia mainly characterized by proximal weakness of the lower extremities with brisk reflexes and spastic gait abnormalities. No additional neurological symptoms were present. After excluding linkage to 12 known HSP loci (*SPG3A, SPG4, SPG6, SPG8, SPG10, SPG12, SPG13, SPG17, SPG19, SPG29, SPG31*, and *SPG33*), a genome-wide screen provided evidence of linkage between the disease and D3S1744 on chromosome 3q (LOD=4.2). Analysis of 6 additional microsatellite markers on chromosome 3q gave significant pairwise LOD score >3 (maximum two-point LOD score 5.07 at recombination fraction 0.001). Therefore, our results clearly establish the existence of a novel locus for autosomal dominant uncomplicated HSP on chromosome 3q24-q26. Haplotype construction and analysis of recombination events narrowed the locus to a 22-cM interval flanked by D3S2326 and D3S3053. This new locus was named *SPG40*. More than 130 genes are located in this region. Sequencing of five candidate genes (*PFN2, SCHIP1, SLITRK3, MYNN, CLDN11*) in this region showed no disease-causing mutation.

The Mexican Genome Diversity Project: Analysis of genetic structure in Mexican Mestizo and Amerindian populations. *A. Hidalgo-Miranda, L. Uribe-Figueroa, I. Silva-Zolezzi, J. C. Fernandez-Lopez, J. Estrada-Gil, G. Ortiz-Ramos, R. Mojica-Espinosa, J. Cruz-Colin, A. Contreras, H. Hernandez-Lemus, S. March, G. Jimenez-Sanchez* National Institute of Genomic Medicine, Mexico.

Most Mexicans are Mestizos resulting from admixture of Amerindian, Spaniard and African populations. The admixture process has led to particular genomic ancestry structure. To optimize the use of human genome information to improve healthcare in Mexicans, we are evaluating genomic variability of the Mexican population. We genotyped 300 Mestizos from six regions: Guanajuato (GUA); Guerrero (GUE); Sonora (SON), Veracruz (VER); Yucatan (YUC) and Zacatecas (ZAC) using the Affymetrix 100K set. Heterozygosity (HET), Fst and principal component analyses were performed. HET was higher in Mexican subpopulations compared to Asian HapMap groups, SON showing the highest (0.287) and GUE the lowest (0.274). Fst values from 5 of 6 pairwise comparisons between Mestizos (0.006 to 0.019) were higher that those of the Asian groups (Fst=0.007). PCA clustered each HapMap group, and Mestizos showed a wide distribution between CEU and other source of variation not present in HapMap. To demonstrate that this ancestral component in Mestizos is Amerindian, we included 33 Zapotec (ZAP) Amerindian samples in the analyses. Fst values showed that GUE and VER are the most similar to the Amerindian sample (ZAP-GUE 0.032; ZAP-VER 0.038) and SON the most different (ZAP-SON 0.082). Fst values for the SON-ZAP comparison were higher than all comparisons between Mestizos and CEU or Asians. As expected, the highest Fst value was obtained in the African-Amerindian comparison (ZAP-YRI 0.238). PCA analysis showed that the ZAP clustered between the CEU and the Asian population. Mestizos from GUE and VER were close to the ZAP cluster, while samples from SON were closer to the CEU. Our results indicate significant heterogeneity between populations within Mexico, as well as between Mexicans and HapMap populations. We are including additional Mexican Amerindians and increasing SNP density to better understand the admixture process in Mexicans, and develop more suitable tools to analyze the genetic bases of complex diseases in this population.

Functional polymorphisms in promoter region of serotonin transporter gene are associated with NEO agreeableness domain. *C. L. Muller[1], M. E. Garrett[1], A. L. Collins[1], B. H. Brummett[2], I. C. Siegler[2], R. B. Williams[2], A. E. Ashley-Koch[1]* 1) Ctr for Human Genetics, Duke University Medical Center, Durham, NC; 2) Department of Psychiatry and Behavioral Sciences, Duke University Medical Center, Durham, NC.

The 5HTTLPR functional polymorphism in the promoter region of the serotonin transporter gene has been previously associated with personality and other psychiatric traits. However, the expression of the serotonin transporter is affected not only by the 5HTTLPR polymorphism but also SNP rs25531 which resides in this region. Composite promoter genotypes may be constructed as high or low expressing based on the combined genotypes of these two polymorphisms and previously established expression levels from each genotype, resulting in three possible genotypes for analysis (HiHi, HiLo & LoLo). In light of the newer information regarding the promoter expression, we examined the factor scores of 5 NEO domains (Neuroticism, Extraversion, Openness, Agreeableness, and Conscientiousness) for association with the composite serotonin transporter polymorphism in a sample of 341 individuals (62.8% female, 63.6% Caucasian) from 162 families. Our sample consists of 297 sibling pairs (126 2-sibling pairs, 12 3-sibling pairs, 1 4-sibling pair, and 1 5-sibling pair) and 23 singletons ascertained on the basis of the Cook Medley Hostility scale (Ho), as well as 21 parents. 59.4% of subjects were classified as low hostile (Ho: 0-9), 19.7% were intermediate hostile (Ho: 10-13), and 20.9% were high hostile (Ho: 14-20). Using the QTDT, we observed that the factor score for the agreeableness domain is highly associated with the composite 5HTTLPR polymorphism ($p<0.0001$) such that individuals with the LoLo genotype are less agreeable than those with either the LoHi or the HiHi genotype. This observation is consistent with previously findings in another data set that show association between the short 5HTTLPR variant (less uptake) and lower agreeableness (Greenberg et al. 2000).

Genome-wide associations for serum bilirubin in the Framingham Heart and Rotterdam studies. *A. D. Johnson[1], M. Kavousi[2], M. H. Chen[1], A. Dehghan[2], C. van Duijn[2], A. Uitterlinden[2,3], A. Hofman[2], F. Rivadeneira[2,3], J. P. Lin[4], L. A. Cupples[5], Q. Yang[1,5], C. J. O'Donnell[1], J. Witteman[2]* 1) NHLBI's Framingham Heart Study; 2) Dept of Epidemiology, Erasmus Med Center, Rotterdam; 3) Dept of Internal Medicine, Erasmus Med Center, Rotterdam; 4) NHLBI's Office of Biostatistics; 5) Department of Biostatistics, Boston University.

Purpose: Variation in serum bilirubin has been associated with altered cardiovascular disease risk and drug metabolism. We aimed to identify genetic contributors to variability in serum bilirubin levels by combining results from high density genome-wide association studies in two white populations. Methods: Genotyping of Framingham Heart Study (FHS) participants was performed with Affymetrix 500K and 50K gene focused arrays and in the Rotterdam Study with Illumina HumanHap550 arrays. Both studies used MACH to impute 2.54 million SNPs based on HapMap CEU phased haplotypes (build 22). Both studies ran age, sex and similar multivariable adjusted models to derive residuals for log- transformed bilirubin levels in FHS Offspring cohort exam 1 (n=3,270) and Rotterdam participants (n=3,847). Imputed genotypes were then tested for association using a linear mixed effects model. We defined 16 a priori candidate loci based on bilirubin physiology. Inverse variance and sample size weighted meta-analysis was conducted using METAL. Results: The UGT1A1 locus was strongly associated with bilirubin levels with minimal p-values 8.8*10-159 and 2.2*10-190, for FHS and Rotterdam, respectively. No other regions exceed a genome-wide significance threshold of 5*10-8 in either study. In meta-analysis a number of loci showed convincing evidence for association, including the UGT locus ($p<5*10-324$) and one of the a priori candidates ($p<1.7*10-10$). Conclusions: In a high density genetic scan and meta-analysis for bilirubin levels across two large cohorts we confirmed the UGT1A1 locus as the major genetic contributor to bilirubin levels, accounting for a large proportion of the variation. We confirm in meta-analysis at least one other locus with strong evidence that influences bilirubin levels. These findings merit further study in independent populations.

High resolution DNA copy number analysis detects previously unreported alterations in human cancer cell lines.
L. Uribe-Figueroa, A. Hidalgo-Miranda, C. Rangel-Escareño, G. Ortiz-Ramos, V. Espinosa-Mateos, R. Goya-Ogarrio, J. Cruz-Colin, R. Mojica-Espinosa, M. Rodriguez-Dorantes, S. March, G. Jimenez-Sanchez National Institute of Genomic Medicine, Mexico.

DNA copy number alterations represent a hallmark of transformed cells. The exact mapping of these aberrations is thus crucial for the identification of genes with a role in cancer development. Several methods have been developed to detect such alterations. Recently, SNP arrays have increased their coverage to evaluate more than 1,800K genetic markers along the genome, including both SNP and non polymorphic copy number variation probes. We compared two SNP microarray platforms to evaluate their capability to detect DNA copy number aberrations in 15 cancer cell lines from colon (HCT15, COLO205), uterine cervix (HeLa, C33A), breast (HS578T, MCF7), leukemia (HL60, K562), lymphoma (RAJI), rhabdomyosarcoma (RD, SMS, RH30), Ewing's sarcoma (A673, HS863T), and liver (Hep-G2) along with three normal lymphoblastoid cell lines (GM1715A, GM17231A and GM17263). All cell lines were analyzed with the 500K and the SNP 6.0 Affymetrix arrays. The copy number analysis was performed using Partek Genomics Suite under the same segmentation conditions for both arrays. DNA copy number aberration patterns were similar in both platforms, considering their differences in genome coverage. The 500K array identifed 3,253 segments with DNA copy number changes, while the SNP 6.0 identified 11,300. Between the most common alterations detected by the SNP 6.0 and missed by the 500K, we found genes associated with carcinogen detoxification, such as GSTT1 (deleted in 4 and amplified in 13 of 18 cell lines) or cell cycle regulation genes, like SCAPER, (deleted in 9 and amplified in 4 cell lines). The higher probe density in the SNP 6.0 allowed the detection of previously unreported copy number alterations in these cell lines, as well as the elimination of false positive copy number calls in regions with low probe coverage in the 500K microarray set. Detection of novel genomic alterations in commonly used cellular models of human cancers will allow a more precise identification of genes involved in cancer biology.

Identifying genes affecting normal variation in human facial features using admixed populations. *D. K. Liberton[1], K. A. Matthes[1], B. McEvoy[2], R. Pereira[3], T. Frudakis[4], M. D. Shriver[1]* 1) Department of Anthropology, Pennsylvania State University, University Park, PA; 2) Queensland Institute of Medical Research, Brisbane, Australia; 3) Programa de Pós-Graduação em Ciências Genômicas e Biotecnologia, Universidade Católica de Brasília, Brasilia, Brasil; 4) DNAPrint Genomics, Sarasota, FL.

Seven selection-nominated candidate genes (*COL11A1*, *LMNA*, *FGFR1*, *FGFR2*, *TRPS*, *BRAF*, *FLNA*) known to be involved in Mendelian craniofacial dysmorphologies and to have high allele frequency differences between West African and European populations were tested for admixture linkage to normal facial feature traits. The sample consists of 254 subjects (n=131 African Americans, n=123 Brazilians) of West African and European genetic ancestry. Each individual was genotyped at 176 ancestry informative markers (AIMs), which allowed for proportional estimation of genetic ancestry from four parental populations and adjustments for admixture stratification.

3D images of faces were acquired using the 3dMDface imaging system. 3D coordinate data were collected from 22 landmarks placed on each image using the 3dMDPatient software. The 231 possible pairwise landmark distances were scaled to the geometric mean and then analyzed using Euclidean Distance Matrix Analysis.

We used both ANOVA and ADMIXMAP to control for admixture stratification and to test for associations between the 231 pairwise landmark distances and 183 AIMs, using sex, height and BMI as covariates. We used a four-population model (West African, European, East Asian, and Native American).

There is a strong concordance between the ANOVA and ADMIXMAP results. Many landmark distances, particularly on the mouth and nose, were significantly associated with genetic ancestry. Additionally, three of the candidate genes show no effects on pairwise landmark distances while four show distinct patterns of association. For example, *FGFR2* is associated primarily with the length of the face. These results represent the first identification of the first genes affecting normal variation in facial features.

Loeys-Dietz syndrome (LDS). In vitro studies of skin fibroblasts showing differences between mutations in the *TGFBR1* and *TGFBR2* genes. *D. Chitayat[1,2], C. P. Barnett[2], T. J. Bradley[3], A. Hinek[4]* 1) Prenatal Diag & Medical Gen, Mount Sinai Hosp, Toronto, ON, Canada; 2) Division of Clinical and Metabolic Genetics, The Hospital for Sick Children, Toronto, Ontario, Canada; 3) Division of Cardiology, The Hospital for Sick Children, Toronto, Ontario, Canada; 4) The Heart Centre, The Hospital for Sick Children, Toronto, Ontario, Canada.

LDS is a newly described condition caused by mutations in the genes encoding transforming growth factor-beta (TGF-) receptors 1 and 2. The condition is associated with vascular tortuousity and formation and rupture of arterial aneurysms. Skin fibroblasts were cultured from 3 patients with confirmed LDS. Case 1 had dilated aortic root and tortuous aortic arch and branches. DNA analysis revealed a *TGFBR1* gene missense mutation (c.722C>T). Case 2 is a 10 year old girl with dilated aortic root requiring surgical repair at age 10, extensive arterial tortuosity of head and neck vessels and a repaired cleft palate. DNA analysis revealed a missense mutation of *TGFBR1* (c.1460G>A). Case 3 had arachnodactyly, bilateral inguinal hernias, bilateral clubfeet and joint laxity. Echocardiography revealed a dilated aortic root. A missense mutation of *TGFBR2* (c.1583G>A) was identified. *In vitro* studies of skin fibroblasts from these patients indicated that both patients carrying mutations of *TGFBR1* demonstrated a significant deficiency in the net expression of elastin and fibrillin genes (assessed by RT-PCR) and did not deposit elastic fibers in primary cultures. In contrast, they produced normal levels of auxiliary components of elastic fibers (fibulins 1, 2 and 5) and deposited normal collagen fibers. Interestingly, fibroblasts derived from patients with mutation of *TGFBR2* genes produced normal components of elastic fibers, but displayed intracellular retention of collagen type 1 and had significantly lower deposition of mature collagen fibers. Our findings indicate that the clinical manifestations associated with *TGFBR1* and *2* mutations, although similar, are caused by different mechanisms.

Knock-out of mouse Ikbkap gene leads to embryonic lethality that can be rescued by human IKBKAP gene. *Y. Chen, M. Hims, R. Shetty, J. Mull, L. Liu, M. Leyne, S. Slaugenhaupt* Ctr Human Genetics Res, Massachusetts General Hosp, Boston, MA.

Tissue specific reduction of IKAP, also known as Human Elongator Protein 1 (hELP1), leads to familial dysautonomia (FD), a devastating hereditary sensory and autonomic neuropathy. All FD patients have an intronic mutation in the IKBKAP gene that disrupts normal mRNA splicing and leads to reduced IKAP protein, particularly in the nervous system. In order to better understand the role of this gene during development, an Ikbkap knockout mouse model was created. Ablating Ikbkap leads to embryonic lethality, with no homozygous Ikbkap knockout embryos (Ikbkap-/-) surviving beyond 10.5 days post cotium. Morphological analyses of the Ikbkap-/- embryos at different stages reveals abnormalities in both the visceral yolk sac and the embryo, including stunted extraembryonic blood vessel formation, delayed entry into mid-gastrulation, disoriented dorsal primitive neural alignment, and failure to establish the embryonic vascular system. IKAP is required for efficient transcriptional elongation of a variety of genes, and we demonstrate down-regulation of several genes that are important for neurulation and vascular development in the Ikbkap-/- embryos. Finally, we show that the embryonic lethality resulting from Ikbkap ablation can be rescued by a human IKBKAP transgene.

Linkage to FEB1 in a Colombian family with autosomal dominant epilepsy with febrile seizures plus, (ADEFS+).

N. Pineda-Trujillo[1,2], J. Tejada-Moreno[1,3], M.-A. Caro-Gomez[1], D. Cabrera[1,2], G. Bedoya[4], A. Ruiz-Linares[4,5], A. Franco[6], C. Castillo-Gomez[1,2], W. Cornejo[1,2], J. Carrizosa[1,2] 1) Mapeo Genético, Departamento de Pediatría y Puericultura, Facultad de Medicina, Universidad de Antioquia, Medellín-Colombia; 2) Pediaciencias, Departamento de Pediatría y Puericultura, Facultad de Medicina, Universidad de Antioquia, Medellín-Colombia; 3) Escuela de Biología, Universidad Industrial de Santander, Bucaramanga-Colombia;; 4) GENMOL, Universidad de Antioquia, Medellín-Colombia; 5) Department of Biology, University of London, UK; 6) Hospital San Vicente de Paúl, Medellín-Colombia.

Febrile seizures (FS) is a clinical and genetically heterogeneous disorder. FEB1 was the first genetic locus associated with the disease. No replication of such association has been reported. Our purpose was to test for linkage to the loci FEB1 and FEB2 in a Colombian family with ADEFS+. Markers D8S533-D8S1795-D8S1807-D8S279 were tested in FEB1; markers D19S219-D19S1034-D19S427-D19S177 were tested in FEB2. These markers were tested by fluorescent methods in a genetic analyzer ABI-310 (Applied Biosystems). Linkage analysis was done using the package LINKAGE v5.1. Several penetrance values were used (0.50, 0.65, 0.90); two phenocopies rate were also considered (0 and 3%). Autosomal dominant inheritance, mutated allele frequency= 0.003 and equal allele frequency for the marker loci were assumed. Haplotype analysis was done with SimWalk2. The highest lod score (Z = 0.75) was obtained at =0, for marker D8S533 (FEB1). In addition, we found a haplotype consistently segregating with the disease, which is characterized by alleles 3-4-4-3 at marker loci in FEB1. These results were obtained assuming penetrance= 0.90 and phenocopies rate= 0. Locus FEB2 did not show linkage to the disorder in this family. Our data support linkage of ADEFS+ to FEB1.This study reports for the first time a replication of the association of the locus FEB1 with febrile seizures (reported by Wallace, et al. 1996). In order to increase power, additional family members are been enrolled in this study. No gene associated with ADEFS+, in this region, has been identified yet. Financial support from Colciencias, grant # 111534319158.

Replication of Associations between Parkinsons Disease and Haplotypes for Iron-Related Candidate Genes. *S. L. Rhodes[1], C. C. Bandong[1], J. M. Bronstein[2], J. C. Lambert[3], J. S. Sinsheimer[4], A. Elbaz[3], J. I. Rotter[1], K. D. Taylor[1], B. Ritz[5]* 1) Medical Genetics Institute, Cedars-Sinai Medical Center, Los Angeles, CA; 2) Dept of Neurology, School of Medicine, UCLA, Los Angeles, CA; 3) National Institute of Health and Medical Research, Institut Pasteur de Lille, Lille, France; 4) Dept of Human Genetics, UCLA, Los Angeles, CA; 5) Dept of Epidemiology, UCLA, Los Angeles, CA.

Postmortem and imaging studies have observed increased levels of iron deposits in parkinsonian vs. control brains, particularly in the substantia nigra, and linked extent of deposit to severity of disease. We investigated associations between Parkinsons disease (PD) and haplotypes for iron-related genes. Stage 1: 372 incident cases and 360 population-based controls from central CA. Stages 2a and 2b: a case-control study of French farmers and the NINDS dbGAP GWAS dataset. Stage 1 candidate genes identified from published literature and prior studies of PD, and tagSNPs selected from Caucasian HapMap data. Stage 2a genotyped specific SNPs from Stage 1, and Stage 2b used imputation to construct comparable haplotypes. Odds ratios (OR) and 95% confidence intervals were estimated adjusted for age, gender, education, and smoking. Of the 90 Stage 1 SNPs, 11 had dominant model p-values <0.05 and 5 had p-values <0.1; for suggestive associations in 10 of 17 genes. We observed protective (0.7, 0.54-0.95) and suggestive risk (1.3, 0.91-1.79) haplotypes in HFE while the frequently and inconclusively investigated 282Y-carrying haplotype was not associated (0.9, 0.58-1.43) with PD. In TF, we observed two protective haplotypes (ORs 0.7, p<0.05); one promoter and one 3 coding block. While Stage 2a and 2b replications are in progress, we have completed the analysis of TF and confirmed a similar association with PD for the promoter haplotype (0.7, 0.48-0.99) but not the 3 coding region haplotype. TF promoter region variants have been associated with variation in total iron binding capacity. Our data suggest associations with PD for iron-related genes and demonstrates the utility of a haplotype tagging approach in candidate gene investigations when the causal variant is unknown.

Application of in-house array-CGH for investigation and diagnosis of congenital genomic disorders. *S. Hayashi, S. Honda, I. Imoto, J. Inazawa* Dept Molecular Cytogenetics, Tokyo Medical & Dental Univ, Tokyo, Tokyo, Japan.

Our purpose is to investigate a cause of a congenital disorder by exploring copy-number aberrations (CNAs) responsible for such disorders and to establish a system for diagnosis of multiple congenital anomalies with mental retardation (MCA/MR) using array-CGH. We formed a consortium with 20 hospitals or universities to recruit patients with MCA/MR, and screened their CNAs using several types of in-house bacterial artificial chromosome (BAC)-based array-CGH. All of the patients had no cytogenetic abnormality in conventional karyotyping. In primary screening we used MCG Genome Disorder (GD) Array containing BAC clones covering loci associated with known genomic disorders and subtelomeric regions of all chromosomes except short arms of acrocentric chromosomes, and detected CNAs in 39 of 378 cases (10.3%). In secondary screening we employed MCG Whole Genome Array-4500 harboring 4523 BACs throughout human genome for cases without any CNAs by GD Array, and detected CNA in 20 of 75 cases (26.7%). Based on the result, we created a database to clarify a correlation between genotype and phenotype and to identify a new disorder. Consequently, we identified the CASK gene as a candidate gene corresponding to X-linked recessive mental retardation in a female patient with heterozygous deletion at Xp11.3p11.4. We also suggested a possible new microdeletion syndrome as a result of analyses of two patients having same heterozygous deletion. This research has provided not only an efficient analysis of MCA/MR but also important clues to investigate each disorder. In conclusion, array-CGH is a useful strategy to investigate cryptic genomic aberrations responsible for unknown MCA/MR, and/or to establish a new syndrome.

Identification of novel genetic regulatory networks in Papillary Thyroid Carcinoma using information theoretical methods. *E. Hernandez-Lemus[1], D. Velazquez-Fernandez[1,2], J. K. Estrada-Gil[1], I. Silva-Zolezzi[1], M. F. Herrera-Hernandez[2], G. Jimenez-Sanchez[1]* 1) National Institute of Genomic Medicine, Mexico; 2) National Institute of Medical Sciences, Salvador Zubiran, Mexico.

Papillary thyroid carcinoma (PTC) is the most frequent endocrine neoplasia worldwide, insight about its molecular pathogenesis has been gained through gene expression (GE) analysis. An integrative approach of this information is still missing. Construction of genetic regulatory networks (GRN) can help understand the interplay between thousands of genes. Three main issues arise in the analysis of this data: the measurement process generates noisy signals, there are more variables involved than experimental samples, and the nonlinear character of the underlying biochemical dynamics is another source of complexity. To overcome some of these limitations, we generated an optimized tool based on the Maximum Entropy Formalism to deconvolute a GRN based on the most probable distribution of gene-gene interactions. We tested the method using GE data from G7 PTC and goiter samples analyzed with the Affymetrix HgU133 Plus 2.0 array. The optimal regulatory network was obtained from a pool of 25,593,993 probability distributions. The interactions were validated by several (mostly in silico) means. The associated PTC-GRN possessed a power-law degree distribution in which the vast majority of the genes have very few links whereas a small number of them are highly connected. This scale-free property has been recognized as characteristic of topological robustness. PTC-GRN is composed by 75 nodes and 170 links, with connectivity k values between 1 and 20, and average connectivity <k> = 2.2666 while the expected average for a random network will be <k_exp> = 2L/N = 4.5. By means of a complex network statistical analysis, we discover some regulatory interactions among genes previously related to PTC including *CTNNB1, AURKA* and *TDG*. *RXRG, KRT19* and *MAP3K10* in the RTK/RAS/RAF/MAPK pathway were also found in the highly correlated gene clusters. These approaches can be of benefit for the identification of new molecular mechanisms of disease.

SLC9A9 is associated with DSM-IV hyperactive-impulsive and total symptoms in AD/HD families. *A. E. Ashley-Koch[1], C. A. Markunas[1], K. Quinn[1], A. L. Collins[1], M. E. Garrett[1], S. Keatts[1], A. M. Lachiewicz[2], E. Morrissey-Kane[3], S. H. Kollins[4], A. D. Anastopolous[3]* 1) Center for Human Genetics, Duke Medical Center, Durham, NC; 2) Dept of Pediatrics, Duke Medical Center, Durham, NC; 3) Dept. of Psychology, University of North Carolina, Greensboro, NC; 4) Dept. of Psychiatry, Duke Medical Center, Durham, NC.

A family was identified that co-segregates a pericentric inversion, 46N inv(3)(p14:q21), with an early-onset behavioral/developmental condition, characterized by impulsive behavior and intellectual deficit (de Silva et al. 2003). The inversion breakpoints lie within intron 19 of the dedicator of cytokinesis 3 (DOCK3) and intron 13 of the solute carrier family 9 (sodium/hydrogen exchanger) isoform 9 (SLC9A9) at the p- and q-arm, respectively. Based on this report, these genes were selected as candidates to be evaluated in a family-based AD/HD genetic association study. Analyses were performed on 102 families with at least one child between the ages of 5 and 12 years who met research criteria for AD/HD. Conners Parent (CPRS) and Teacher Rating Scales (CTRS) of AD/HD symptoms were collected on 163 affected and unaffected siblings aged 5-18 years. Parents and children were genotyped using the Illumina Infinium HumanHap300 duo chip and a minimal number of tagging SNPs in each gene (DOCK3: n=10; SLC9A9: n=79) were selected for analysis. Using QTDT, each SNP was tested for association with the t-scores for the Conners DSM-IV subscales: inattentive, hyperactive-impulsive, and total symptom count. Teacher-generated t-scores were log-transformed prior to analysis. After adjusting for multiple testing, one SNP (rs1046706) in the 3 UTR of SLC9A9 remained significantly associated ($p<0.0001$, FDR-adjusted $p<0.001$) with scores on the DSM-IV hyperactive-impulsive and total symptom subscales according to the CTRS. Interestingly, SLC9A1 (member of the SLC9A family) null mice show increased hippocampal neuronal excitability (Gu et al. 2001). These results suggest that SLC9A9 may be responsible for the phenotype observed in the inversion family and that further investigation of the role of SLC9A9 in AD/HD and other behavioral disorders is warranted.

The ITMAT/Broad/CARE (IBC) Candidate Gene Array Identifies Novel Type 2 Diabetes Gene Associations with Coronary Artery Disease: The GRAND-CAD Consortium. *N. N. Mehta[1], M. Li[1], M. S. Burnett[2], N. T. Ginwala[1], A. Qasim[1], S. Restine[1], L. Pruscino[1], I. Stylianou[1], Z. Chen[1], J. He[1], M. L. Wolfe[1], J. Devaney[2], H. Chandrupatla[1], H. Hakonarson[1], B. Keating[1], J. Lindsay[2], R. Waksman[2], S. E. Epstein[2], D. J. Rader[1], M. P. Reilly[1]* 1) University of Pennsylvania, Philadelphia, PA; 2) Cardiovascular Research Institute/MRI/Washington Hospital Center, Washington, DC.

Patients with type 2 diabetes (T2D) represent a high risk population for coronary artery disease (CAD). Many candidate genes have emerged from recent genome wide association studies (GWAS) of T2D. Identifying which T2D genes are associated with increased risk of CAD may provide insight into the complex genetics of T2D and CAD. The IBC array is a custom 50K cardiovascular disease (CVD) gene centric SNP array designed to assess the genetic diversity within candidate genes (~2000) in CVD. Many T2D genes were included based on results of T2D GWAS; we focus on their association with CAD. In stage 1, we examined SNPs in T2D genes [TCF7L2, FTO, PPARG, IGF2BP2, KCNJ11, HHEX, CDKAL1, SLC30A8, 9p21 T2D locus, rs1081161] with CAD in a Caucasian, angiographic case-control study (PennCATH: 1018 CAD cases & 498 controls), with gene based Bonferroni correction. Stage 2 in silico replication was performed in a similar cohort (MedStar: 875 CAD cases & 447 controls) using Affymetrix 6.0 data. All samples & SNPs passed stringent QC; there was no significant population stratification. After adjustment for age, gender, T2D & BMI, we found significant associations in 2 of 9 T2D genes with CAD in stage 1 (CDKAL1 [rs6915037, p=0.0019] & TCF7L2 [rs11196224, p=0.0011]). There was no significant association with the other T2D loci. In stage 2, CDKAL1 (rs946598, p=0.003), but not TCF7L2 (best SNP rs7094463, p=0.04) replicated the association with CAD. Conclusion: We have identified & replicated a significant association with CAD for novel T2D genes. This CAD association appears to be independent of T2D suggesting a novel genetic mechanism modulating both T2D and CAD. Knowledge of these associations may facilitate the development of targeted risk prediction and therapy for CAD in T2D.

Effect of PNA based inhibitor on microRNA activitiy. *S. Y. Oh, H. Park* Panagene, Daejeon, Korea.

The microRNAs are approximately 22 nucleotides non-coding RNAs and are transcribed from DNA as hairpin precursors. They regulate major processes as development, apoptosis, cell proliferation, hematopoiesis, and patterning of the nervous system. miRNA inhibition using antisense oligonucleotide is unique and effective technique for miRNA functionalization and therapeutic targeting. Peptide nucleic acids (PNAs) are artificial oligonucleotides with a peptide backbone. PNAs have stronger affinity and greater specificity than DNA oligonucleotides for binding to DNA and RNA. Also, PNAs are resistant to nuclease, which is essential for a miRNA inhibitor that be exposed to abundant serum and cellular nucleases. These properties make PNAs well suited for miRNA inhibitor. We described here a new PNA inhibitor and evaluated effect of PNA inhibitors on miRNA activity. In this experiments, we confirmed that miRNA inhibition effects of PNA is over two times higher than LNA-modified DNA and 2-O-Methyl-oligonucleotide (2-OMe) by luciferase assay and northern blot. For confirmation for PNA inhibitor effects, we also observed that bcl-2 expression which is regulated by miR16 was decreased by western blot. In addition, the effect of PNA inhibitors on miRNA is active after 10 days of transfection. These results demonstrated that PNAs are more powerful and effective miRNA inhibitor than LNA-modified DNA and 2-OMe.

Absence of Association of Y1102 and C4848T variants with sudden cardiac death among African-Americans. *R. M. Faugue[1], Y. Rodriguez[1], D. El-Imam[1], ME. Ahearn[1], E. Mont[3], R. J. Myerburg[2], N. Bishopric[1]* 1) Molecular and Cellular Pharmacology , University of Miami Miller School of Medicine, Miami, FL; 2) 2Division of Cardiology, University of Miami Miller School of Medicine, Miami, FL; 3) Miami-Dade County Medical Examiner Department, Miami, FL.

BACKGROUND. Mutations in the cardiac sodium channel gene (SCN5A), such as Y1102 and C4848T, have been proposed to be over-represented in populations of AA patients being followed in an arrhythmia clinic. The same Y1102 variant was also associated with unexplained or hypertrophy-associated sudden death in 65 cases referred to the Maryland Medical Examiner. Y1102 amino acid substitution has been reported to slightly prolong Na current during repolarization phase of the cardiac action potential and enhanced sensitivity due to the use of anti-arrhythmic drugs such as amiodarone, as well as unexplained sudden cardiac death. We sought to confirm this finding or identify novel polymorphic variants in arrhythmia-associated genes in a population of sudden cardiac death victims who had post-mortem examinations by the Miami-Dade Medical Examiner. METHODS. We investigated the incidence of the Y1102 and 4848 (C>T) alleles in a series of sudden death in AA subjects. This ME group was suspected of having LQTS or unexplained arrhythmias. Most of these cases of sudden cardiac death were shown to have underlying coronary artery disease, others did not have any cardiac pathology. They were compared to a control group of 72 AA. 72 AA probands were analyzed using Custom TaqMan SNP Genotyping Assays using an ABI 7900HT Fast Real-time PCR and a novel Xmn I restriction enzyme digestion assay (New England BioLabs). RESULTS. The overall frequency of Y1102 was 15% of probands and 16% of controls. The results were not significant ($p = 0.15$). Similarly, the 4848 (C>T) SNP overall frequency was 24/72 probands (33%) and 20/50 controls (40%). Both Y1102 and 4848 results were not statistically different. CONCLUSION. We were unable to find an association between the Y1102 SNP and 4848 SNP in sudden death cases of AA. Differences in ethnicities may contribute to genetic variation but it is difficult to assign a risk in such a diverse population.

Mutations in patched domain containing 1 gene (PTCHD1) are associated with autism spectrum disorder. *A. Noor[1], C. R. Marshall[2], S. W. Scherer[2], J. B. Vincent[1]* 1) Neurogenetics Section, Centre For Addiction & Mental Health, Toronto, ON, Canada; 2) Program in Genetics and Genomic Biology and The Centre for Applied Genomics, The Hospital for Sick Children, Toronto, ON, Canada.

In a study of genomic variants in autism spectrum disorder using high-density single nucleotide polymorphism (SNP) microarrays, we have identified a 167 Kb deletion in a male proband and his affected male sibling, that results in the deletion of exon 1 and upstream regulatory regions of the patched domain containing 1 (PTCHD1) gene on Xp22.11. The presence of the patched domain suggests a possible role for PTCHD1 in the Hedgehog (Hh) signaling pathway. We sequenced the entire coding region of this gene in 900 unrelated autism probands, and identified six families with PTCHD1 DNA sequence variants resulting in substitution of amino acids at highly conserved positions. All these variants were inherited from unaffected, heterozygous mothers. In two cases, these apparently deleterious variants accompanied de novo CNVs elsewhere in the genome suggesting in these individuals two or more variants may be required for phenotypic expression. The PTCHD1 deletion was not present in 1652 control individuals and the missense changes were not present in >300 controls. We confirmed expression of this gene in various human tissues, including adult and fetal brain. Localization studies revealed that PTCHD1 fusion-protein is predominantly localized in the cell membrane. Our findings suggest that loss of function or expression of PTCHD1 contribute to the etiology of Autism Spectrum Disorder (ASD). Furthermore, our findings demonstrate a possible contribution of more than one loci in the etiology of Autism, thus, supporting the oligopolygenic inheritance of this disorder.

Bohring-Opitz Syndrome or C Syndrome spectrum? Report of a Brazilian case. *F. B. Piazzon*[1,3], *M. F. F. Soares*[2], *V. F. A. Meloni*[1] 1) Centro de Genética Médica, Universidade Federal de Sao Paulo - UNIFESP, Sao Paulo, Sao Paulo, Brazil; 2) Departamento de Diagnóstico por Imagem, Universidade Federal de Sao Paulo - UNIFESP, Sao Paulo, Sao Paulo, Brazil; 3) Instituto de Genética e Erros Inatos do Metabolismo - IGEIM / UNIFESP, Sao Paulo, Sao Paulo, Brazil.

Introduction: Bohring et al. [1999] reported a new syndrome in 4 unrelated patients with specific features. Two prior, very similar cases with this new entity were reported in 1975 by Oberklaid and Danks then described as Opitz trigonocephaly (OMIM #211750). The new syndrome consisted of bulging forehead over the metopic suture, frontal nevus flammeus, hypertelorism, exophthalmos or even prominent eyes, upslanting palpebral fissures, and cleft palate and/or lip, as well as flexion deformities of the upper limbs, multiple other anomalies, and severe failure to thrive. In 2000, more cases were characterized and Bohring-Opitz was chosen to name this syndrome (OMIM #605039). **Objectives:** to describe a 6-month-brazilian girl with the present diagnostic hypothesis. **Materials and methods:** clinical follow-up with anamnesis and physical examination; subsidiary exams and photographic documentation. **Results:** in the first appointment the girl was 2 months old, an investigation for the trigonocephaly was initiated; cleft palate, cardiac murmur and other dysmorphic features were also present. The patient had microcephaly with trigonocephaly, exophthalmos, upslanting palpebral fissures and typical deformities on her hands and feet. Global development was delayed. She was fed through a G-tube, which strongly contributed to the failure to thrive. **Conclusion:** Because of close similarity between both syndromes, Bohring-Opitz syndrome is considered the more severe form of the Opitz trigonocephaly (C syndrome), therefore known as C-like syndrome. This similarity became more plausible, after the discovery of the involvement of a mutation in the *TACTILE* gene, located at 3q13.3, which encodes the CD96 immunoglobulin, in Bohring-Opitz or C Syndrome patients, that is why it has been suggested that both syndromes can be part of the same clinical spectrum of Opitz trigonocephaly.

Expression of miRNAs on Chromosome 11q in HNSCC. *B. Henson[1], S. Bhattacharjee[1], S. Gollin[1,2]* 1) Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA; 2) University of Pittsburgh Cancer Institute, University of Pittsburgh, Pittsburgh, PA.

MicroRNAs (miRNAs) are a class of small non-protein encoding RNAs that can inhibit translation by binding to the 3` UTR of target mRNAs and either sequester or degrade the transcript. Altered miRNA expression profiles have been found in numerous malignancies. Head and Neck Squamous Cell Carcinoma (HNSCC) is the sixth most common cancer in the United States; however, little is known about its miRNA expression profile and how this affects the disease. Numerous genetic aberrations are characteristic of HNSCC, with the amplification of chromosomal band 11q13 and loss of distal chromosome 11q being amongst the most prevalent. Many of the protein-encoding genes within these regions and their contributions to HNSCC have been examined. Our goal was to determine expression levels of miRNAs on chromosome 11q using RT-PCR and determine if modifying their expression affects cell proliferation (MTT assay), cell cycle progression (flow cytometry), and global gene expression (microarray analysis). We hypothesize that expression of miRNAs on chromosome 11q is decreased in conjunction with distal 11q loss and that modifying their levels will affect cellular behavior. We found that mir-125b and mir-100 levels were significantly lower in HNSCC cell lines and tumors than in controls. Increasing levels of mir-125b and mir-100 had a marginal effect on the cell cycle, but significantly decreased cell proliferation and altered global gene expression. Microarray analysis revealed that the expression levels of predicted miRNA target genes and non-target genes were significantly modified. Altered expression of non-target genes could be due to these genes being unknown targets of these miRNAs; however, it is more likely that their expression is modified via an indirect mechanism. Both mir-125b and mir-100 have altered expression levels in other malignancies and each has the capability to regulate genes involved in many vital biological functions. Thus, both have enormous potential as therapeutic agents in HNSCC as well as other malignancies.

Sign, Sign, Everywhere a Sign: High Density Haplotype Maps of the Dog, Human, and Cow Genomes Reveal Extensive Human Reorganization of Domesticated Genomes. *C. D. Bustamante[1], R. K. Wayne[2], M. Nordborg[3], M. R. Nelson[4], M. Cargill[5], R. A. Gibbs[6], E. A. Ostrander[7]* 1) Cornell University, Ithaca, NY; 2) UCLA, Los Angeles, CA; 3) U. Southern California, Los Angeles, CA; 4) GlaxoSmithKline, Raleigh, NC; 5) Navigenics, Redwood, CA; 6) Baylor College of Medicine, Houston, TX; 7) NHGRI, NIH, Bethesda, MD.

We have developed multi-population high-density SNP and Haplotype maps of the bovine, human, and domestic dog genomes. These maps came about through three separate efforts: (1) the CanMap Project (1,000 dogs and wolves from 85 breeds genotyped across 127K SNPs), (2) the Bovine HapMap project (500 bulls on 25K SNPs from 25 breeds), and (3) the GSK-POPRES project (3,835 humans of diverse ethnic and geographic origin genotyped on Affy 500K human arrays). Using phased-resolved haplotype maps, we estimate local population recombination rate along each chromosome, identify key determinant of population substructure, and develop maps of recent directional selection for each species. By comparing the recombination maps of the three species, we find that humans show a very high correlation at the megabase scale in estimated population recombination rates across subpopulations; however, cows and domestic dogs show a striking lack of correlation across breeds. We also find that dogs and cattle show pervasive signatures of recent selection using SNP and haplotype-based statistics. Simulations suggest that, surprisingly, domestication bottlenecks do not explain these patterns. Strong bottlenecks at the time of breed formation coupled with popular sire effects, on the other hand, are necessary. We also find that all breeds examined demonstrate high degrees of cryptic relatedness, even when close relatives are avoided at the time of sampling. This implies that great care must be taken in interpreting nominal and genome-wide corrected p-values in whole genome association mapping within domesticated species. Comparison of various algorithms for WGAM in structured populations suggests mixed-model approaches as well as weighted permutation tests may effectively control for the induced background relatedness.

Association study of *GPR74* and *GNB3* gene polymorphisms with obesity in Koreans. *D. Shin[1,3], J. Han[1,4], J. Im[1], S. Lee[1,2], S. Park[1,2], E. Shin[5], J. Lee[5], Y. Jang[1,2,3]* 1) Cardiovascular Genome Ctr, Yonsei Col Medicine, Seoul, Korea; 2) Division of Cardiology, Yonsei University College of Medicine; 3) Yonsei University Research Institute of Science for Aging; 4) Graduated Program in Science for Aging, Yonsei University; 5) DNA Link Inc, Seoul, Korea.

G-protein-coupled receptor 74 (*GPR74*) is a novel candidate gene for body weight regulation, and is predominantly expressed in the brain, heart, and adipose tissue. GPR74 has a high affinity for neuropeptide FF (NPFF), which is responsible for cardiovascular regulation and to have effects on food intake. Heterotrimeric G-proteins are intracellular signal transducers, and C825T polymorphism in the G-protein 3 subunit (*GNB3*) gene has been associated with obesity, hypertension, and diabetes. We carried out a case-control study to investigate how genetic variations in the *GPR74* and *GNB3* genes are associated with obesity in Koreans. We examined a sample population of 624 Koreans, comprising of 312 controls (nonobese subjects, BMI 25) and 312 cases (obese subjects, BMI 25). We analyzed 5 single nucleotide polymorphisms (SNPs) in *GPR74* (C5564T, C15539G, A63920G, A84008G, G105951A) and 3 SNPs in *GNB3* (C825T, C1429T, G5177A). All subjects were genotyped for these polymorphisms by single base primer extension assay using the SNaPShot assay. *GPR74* C5564T and C15539G variants were strongly associated with BMI status between the obese and the nonobese subjects in dominant and codominant models (*P* 0.002). *GPR74* 4 haplotypes were also significantly associated with BMI in dominant and codominant models (*P* 0.001). In analysis of gene-gene interaction, we found that there were significant interaction between the *GNB3* and *GPR74* SNPs (*P* 0.05). Odds ratios (ORs) for obesity were significantly higher in *GNB3* 825T carriers with *GPR74* 15539CC genotype (OR, 3.016; 95% CI, 1.136-8.008; *P* = 0.0232), and in *GNB3* 1429CC genotype with *GPR74* 15539G carriers (OR, 0.429; 95% CI, 0.191-0.963; *P* = 0.0385). These findings suggest that *GPR74* gene polymorphisms are associated with obesity in Koreans and imply that the interaction between *GNB3* and *GPR74* genes has the predisposing or protective effect for obesity.

Paternal Uniparental Disomy as a Mechanism for Inherited Surfactant Deficiency. *D. J. Wegner[1], L. Nogee[2], A. Kammesheidt[3], L. Kasch[2], F. S. Cole[1], A. Hamvas[1]* 1) Pediatrics, Washington University, St. Louis, MO; 2) Pediatrics, Johns Hopkins University, Baltimore, MD; 3) Ambry Genetics, Aliso Viejo, CA.

Introduction: Uniparental disomy (UPD) has not been recognized as a mechanism of genetic disruption of neonatal pulmonary surfactant metabolism. The gene that encodes the ATP binding cassette family member A3 (ABCA3) has been mapped to chromosome 16 (16p13.3), the chromosome most commonly duplicated in cases of placental aneuploidy. Recessively inherited, loss-of-function ABCA3 mutations disrupt surfactant metabolism and function and cause lethal, neonatal respiratory disease. Objective: To determine the frequency of UPD in infants homozygous for recessive loss of function mutations in ABCA3. Methods: We searched datasets from 3 laboratories that perform sequence analysis for ABCA3 for infants with respiratory dysfunction (n=600) to identify those who were homozygous for loss of function mutations and for whom parental DNA was available. For infants with only one heterozygous parent, we used 9 microsatellite markers that span the length of chromosome 16 to characterize allele inheritance. Results: We found 18 infants homozygous for loss of function mutations in ABCA3 and for whom parental DNA was available. We identified 2 cases in which the infant was homozygous for a rare loss of function mutation (K914R and P147L, respectively) and for whom only the father was heterozygous for the respective mutation. One infant (K914R) was homozygous for all 9 markers, 7 of which confirmed paternal isodisomy, while the other infant (P147L) was homozygous for 7 markers, 3 of which were informative for the paternal allele, and heterozygous for 2 markers, a pattern that suggests partial isodisomy (heterodisomy). We identified no extrapulmonary phenotypic abnormalities in either infant. Both underwent lung transplantation at 5 and 3 months of age, respectively, for progressive pulmonary failure. Conclusions: Uniparental disomy is not a rare mechanism of ABCA3 deficiency. Confirmation of parental carrier status is important to provide informed and specific reproductive counseling to families of affected infants.

Microdeletion/duplication at 15q13.2q13.3 among individuals with features of autism and other neuropsychiatric disorders. *D. Miller[1, 2], Y. Shen[2, 3], L. Weiss[3], G. Cox[1], D. Harris[1], R. Hundley[4], R. Nasir[4], A. Reinhard[1, 2], M. Sobeih[5], J. Stoler[1], W.-H. Tan[1], J. Gusella[3], M. Daly[3], B.-L. Wu[2]* 1) Dviision of Genetics, Children's Hospital Boston, Boston, MA; 2) Dept. of Laboratory Medicine, Children's Hospital Boston, Boston, MA; 3) 3. Center for Human Genetic Research and Psychiatric and Neurodevelopmental Genetics Unit, Massachusetts General Hospital, Boston, MA; 4) 7.Division of Developmental Medicine, Children's Hospital Boston, Boston, MA; 5) 5.Department of Neurology, Children's Hospital Boston, Boston, MA.

BACKGROUND: Deletion at breakpoints (BP4-BP5) of chromosome 15q13.2q13.3 is associated with mental retardation, epilepsy, and/or EEG abnormalities. PATIENTS: 1445 consecutive DNA samples from clinical patients at Childrens Hospital Boston and from 751 families with Autism from the Autism Genetic Resource Exchange (AGRE) repository. RESULTS: We report clinical features of four patients with a BP4-BP5 deletion, 3 with a BP4-BP5 duplication, and 2 with an overlapping but smaller duplication identified by whole genome high resolution oligonucleotide array CGH. BP4-BP5 deletion cases exhibit minor dysmorphic features, significant expressive language deficits, and a spectrum of neuropsychiatric impairments that include autism spectrum disorder, ADHD, anxiety disorder, and mood disorder. Cognitive impairment varied from moderate mental retardation to normal IQ with learning disability. BP4-BP5 covers ~1.5Mb (chr15:28.719-30.298Mb) and includes 6 reference genes, while the smaller duplications cover ~500 kb (chr15:28.902-29.404 Mb and contain 3 reference genes. The BP4-BP5 deletion and duplication events span CHRNA7, a candidate gene for seizures. However, none of these individuals reported here has epilepsy, although one has an abnormal EEG. CONCLUSIONS: The phenotype of chromosome 15q13.2q13.3 BP4-BP5 microdeletion/duplication syndrome may include features of autism spectrum disorder, a variety of neuropsychiatric disorders, and cognitive impairment. Recognition of this broader phenotype has implications for clinical diagnostic testing and efforts to understand the underlying etiology of this syndrome.

Tissue specificity of SERPPINB5 promoter in gastric cancer. *M. Kim, H. Ju, B. Lim, C. Kang* Biology, KAIST, Daejeon, Korea.

SERPINB5, a member of the human serpin peptidase inhibitor family, is a multifaceted protein regulating tumor cell homeostasis through inhibition of cell growth, motility and invasion and is also called mammary serine protease inhibitor (maspin) or protease inhibitor 5 (PI5). It was associated with gastric cancer (GC) susceptibility in this case-control association study using 502 unrelated Korean patients with GC and 406 unaffected controls. Four SNPs located in the promoter region were significantly associated with both intestinal- and diffuse-type GC, and risk and non-risk haplotypes were identified. When fused to the luciferase gene, the risk-haplotype promoter had lower activity in well-differentiated GC cell-lines but higher activity in moderately- or poorly-differentiated GC cell-lines, versus the non-risk haplotype promoter. Thus, the SERPINB5 promoter showed specificity with respect to different gastric cell lines, likely due to tissue-specific presence of active transcription factors such as AP-1, AP-2, Ets and/or p53 that is known to bind to the promoter.

Treatment with Dexamethasone Reverses Impared Elastogenesis and Collagenogenesis in Cultures of Fibroblasts from Patients with Loeys-Dietz Syndrome (LDS). *A. Hinek[1], C. P. Barnett[2], T. J. Bradley[3], D. Chitayat[2]* 1) Heart Centre, Hospital for Sick Children, Toronto, Ont, Canada; 2) Division of Clinical and Metabolic Genetics, The Hospital for Sick Children, Toronto, Ontario, Canada; 3) Division of Cardiology, The Hospital for Sick Children, Toronto, Ontario, Canada.

LDS is an autosomal dominant condition caused by mutations in the *TGFBR1* and *TGFBR2* genes. It is associated with facial dysmorphism, vascular tortuosity and life threatening aortic aneurysm. Recent successful use of losartan in the Marfan mouse model has raised hope for medical treatment of LDS. Here we report a promising *in vitro* response in dexamethasone-treated cultured fibroblasts from three LDS patients. Case 1 presented with LDS features and a dilated aortic root at 2 years. DNA analysis revealed a missense mutation of *TGFBR1* (c.722C>T). Case 2 was diagnosed with a dilated aortic root requiring surgical repair at age 10. A missense mutation of *TGFBR1* (c.1460G>A) was found. Case 3 presented in the neonatal period with LDS features and a dilated aortic root. DNA analysis revealed a missense mutation of *TGFBR2* (c.1583G>A). *In vitro* studies of skin fibroblasts from these patients indicated that both patients with mutations of *TGFBR1* demonstrated a significant deficiency in the expression of elastin and fibrillin genes (RT-PCR). In contrast, they deposited normal collagen fibres. Fibroblasts derived from the patient with a *TGFBR2* mutation produced normal elastic fibers, but displayed intracellular retention of collagen type 1 and significantly lower deposition of mature collagen fibers. Addition of 10-5M of DEX to cultured fibroblasts restored normal elastogenesis in cultures of fibroblasts with mutations of *TGFBR1* gene and normalized collagen fiber production in fibroblasts carrying *TGFBR2* gene mutation. Further studies are needed to establish whether DEX can have a therapeutic effect in patients with LDS. Prenatal treatment of affected fetuses may prevent or ameliorate the clinical manifestations of this disorder.

Novel mutations in the HEXA gene and corresponding enzyme activities in non-Ashkenazi Jewish Tay-Sachs carriers. *N. Park[1], C. Morgan[2], R. Sharma[2], Y. Li[1], R. Lobo[2], J. Redman[1], D. Salazar[2], W. Sun[1], J. Neidich[2], C. Strom[1]*
1) Molecular Genetics, Quest Diagnostics, San Juan Capistrano, CA; 2) Biochemical Genetics, Quest Diagnostics, San Juan Capistrano, CA.

Tay-Sachs disease (TSD) is a progressive neurodegenerative disease inherited in an autosomal recessive fashion. It is caused by mutations in the HEXA gene in chromosome 15, which encodes an alpha subunit of the enzyme hexosaminidase A (Hex A). Absence of Hex A activity leads to lysosomal accumulation of undegraded glycolipid, particularly in the nervous system. Individuals of Ashkenazi Jewish (AJ), and non-AJ origin have approximately 1:30 and 1:300 carrier frequency for TSD, respectively. Only 3 mutations account for ~99% of TSD cases in the AJ population: c.1421+1G>C (also known as IVS12+1G>C), c.805G>A (also known as G269S), and c.1277_1278insTATC. Non-AJ TSD patients, on the other hand, have much more diverse mutations across the HEXA gene, and over 90 disease causing mutations have been identified. Therefore, Hex A enzyme analysis is the preferred method for carrier detection in non-AJ population. Here, we present results of HEXA gene DNA sequencing on 30 non-AJ TSD carriers. Our sequencing assay identified mutations in 90% of the samples, and three of these mutations are novel. We also performed sequencing on samples with a "grey zone" enzyme level, and we were able to clarify the carrier status. Our HEXA gene sequencing assay can accurately identify mutations and can be used to clarify ambiguous enzyme results in non-AJ population.

Elucidation of etiologies in complex diseases under multivariate variance component-threshold model. *A. Narita[1], K. Yasuno[1], H. Nakaoka[1], A. Tajima[1], I. Inoue[1,2]* 1) Dept. Molecular Life Science, Tokai University School of Medicine, Isehara, Kanagawa, Japan; 2) Core Research for Evolutional Science and Technology, Japan Science and Technology Agency, Kawaguchi, Saitama, Japan.

Recent remarkable progress in genome science has made it possible to use hundreds of thousands of genetic polymorphisms for genome-wide association studies, and a large number of polymorphisms have been unveiled to be associated with common complex human diseases at a rapid rate. However, as already well known, these diseases are much more likely to be attributed to quite a complicated network, in which not only the polymorphisms but also other non-genetic factors are correlated and/or interacting with each other, rather than the individual effects of these polymorphisms. The aim of the study is to propose an approach to elucidate the etiologies of the multifactorial diseases by combining the multivariate variance component (MVC) model and the threshold model. Since the MVC model treats multilocus genotypes as random effects which have a normal distribution with only two parameters, mean and standard deviation, a relatively high-dimensional interaction can be tested without substantial loss of degrees of freedom, i.e., loss of power. The threshold model, which assumes that an apparently discrete variable is determined by a normally distributed liability and threshold(s), enables both categorical data such as affection status and continuous values, such as age and body mass index, to be analyzed comprehensively as multivariate normally distributed variables. Additionally, using the Markov chain Monte Carlo, the most likely combination of causal factors for a disease is efficiently searched for and missing values can be also imputed. In the study we demonstrate the validity of the proposed method using simulated data sets, and show that it can reach the correct etiological model and also provide unbiased estimates for respective parameters. The approach will be further extended to analyze a real data set, including demographic and/or clinical information, for more reliable diagnosis and prediction of onset.

Clinical and Cytogenetic Characterization of 6 Patients with Deletion 9p Syndrome. *A. Alsaegh, AM. Innes, J. Chernos* Medical Genetics, University of Calgary, Calgary, Alberta, Canada.

We report 6 cases of del (9p) syndrome (3 males and 3 females) that were referred to the Alberta Childrens Hospital from 1996 to 2008. Patients were diagnosed by routine cytogenetic analysis (chromosome karyotype/subtelomeres) and their ages at diagnosis ranged from few days of birth to 4 years. Our patients have the following cytogenetic breakpoints (3 patients with 9p22, 2 patients with 9p24 and 1 patient with 9ptel). The Clinical phenotype in our patients is similar to previously published literature on classical 9p deletion syndrome that was first described by Alfi et al in 1973. Several malformations have been described in del (9p) syndrome, two of our patients presented with rare findings seen in this syndrome, post axial polydactyl (-9p22) and U shape complete cleft palate (-9p24). Cytogenetically, 9p deletion syndrome represents a heterogeneous condition with variable deletion size. Based on previous studies no clear genotype/phenotype correlation could be established for the various features seen in this syndrome. We observe that all our patients with the deletion share the same similar dysmorphic features, however, patients with the del (9) (p22) presented with congenital malformations including trigonocephaly, congenital heart defect, ano-rectal and genital anomalies. This syndrome was rarely suspected prior to the cytogenetic diagnosis. Patients with deletion involving 9p22 have typical dysmorphic features and recognizable congenital anomalies and diagnosis should be suspected at birth.

PKU Treatment with tetrahydrobiopterin (sapropterin) during pregnancy. *G. Pridjian[1, 2], A. Cunningham[2], S. Tafti[1], H. Andersson[2]* 1) Department of Obstetrics & Gynecology; 2) Human Genetics, Hayward Genetics Center, Tulane University School of Medicine.

Tetrahydrobiopterin (BH4) has been shown to significantly reduce the level of plasma phenylalanine (PHE) in 30-50% of PKU patients. The drug was recently FDA-approved for treatment of PKU individuals in conjunction with traditional dietary therapy. Treatment of adult phenylketonuria with BH4 (sapropterin) during pregnancy has not been systematically studied and only one case has previously been reported. In the FDA use in pregnancy ratings, BH4 is classified as Class C because no pregnant animal studies have been conducted and there are no adequate and well-controlled studies in pregnant women.

We report a case of a 29-year old pregnant PKU patient treated with BH4 during the pregnancy. She demonstrated responsiveness to BH4 prior to becoming pregnant. In the preconception period she was counseled regarding the risks and benefits of use of this medication in pregnancy. After counseling, she elected to continue BH4 administration throughout the pregnancy. Mean plasma PHE prior to BH4 administration was 480 mcM (SD=90), substantially higher than the recommended range of 120-360 mcM; after starting BH4, plasma PHE dropped to 210 mcM (SD=36) from 10 weeks prior to pregnancy and throughout pregnancy (week 16 at the time of this abstract) without any dietary modification. The patient did experience anorrhexia and nausea of the first trimester of pregnancy and had decreased caloric intake during this period according to food diaries. Despite endogenous protein catabolism, the plasma PHE value remained normal in the first trimester. The patient has tolerated BH4 well. Second trimester targeted ultrasound has revealed no fetal anomalies or growth abnormalities. Subsequent course, PHE values, ultrasounds, and birth data will be presented.

Implications of absence of clinical phenotype on Morquio A mice: Why rodents do not require skeletal keratan sulfate? *A. Montano[1,2], M. Goldim[1], Y. Satta[2], N. Takahata[2], S. Tomatsu[1]* 1) Dept Pediatrics, St Louis Univ, St Louis, MO,USA; 2) Dept. Biosystems Science, The Graduate University for Advanced Studies (SOKENDAI), Shonan Village, Hayama, Kanagawa, Japan.

Proteoglycans are a set of ubiquitous proteins found on cell surfaces, within intracellular vesicles, and incorporated into extracellular matrices. They have covalently linked to their protein core sulfated glycosaminoglycans such as heparan sulfate, heparin, chondroitin sulfate (CS), dermatan sulfate and keratan sulfate (KS). Cartilage contains heterogeneous populations of aggregating and non-aggregating large proteoglycans relatively enriched in CS or KS. It has been shown that human and bovine cartilage proteoglycans contain skeletal KS (KSII). However, mouse and rat do not synthesize KS II at any developmental stage. The Morquio A (MPS IVA) mouse has little storage material in the cartilage and absence of clinical phenotype while human Morquio A patients have systemic bone dysplasia. To gain insight into the skeletal KS metabolism in mouse and human and to clarify why KS II is deficient in rodents, we have designed subtraction experiments to identify candidate genes involved in this process. We obtained 1363 subtracted cDNA clones. Differential gene expression screening was performed, and nearly hundred genes were found to be over-expressed in either species. Two potential candidate genes involved in KS II synthesis were identified and had homologies with glycosyltransferases. The identification of genes involved in KS II metabolism will shed lights on understanding the biological significance of this glycosaminoglycan in cartilage.

Association testing for type 2 diabetes in FIND families. *A. Malhotra[1], R. Igo[2], F. Thameem[3], W. H. L. Kao[4], H. E. Abboud[3], S. G. Adler[5], B. I. Freedman[6], S. K. Iyengar[2], P. Kimmel[7], W. C. Knowler[1], O. Kohn[8], D. Leehey[9], S. B. Nicholas[10], R. S. Parekh[4], S. S. Rich[11], J. I. Rotter[10], J. R. Sedor[2], V. Shah[12], D. Thornley-Brown[13], P. G. Zager[12], FIND Research Group* 1) NIDDK, Phoenix, AZ; 2) Case Western Reserve U, Cleveland, OH; 3) U of TX, San Antonio, TX; 4) Johns Hopkins U, Baltimore, MD; 5) Harbor-U of CA LA Med Center, Torrance, CA; 6) Wake Forest U, Winston-Salem, NC; 7) NIDDK Program Office, Bethesda, MD; 8) U of Chicago, Chicago, IL; 9) Loyola U, Chicago, IL; 10) U of CA, Los Angeles, CA; 11) U of VA, Charlottesville, VA; 12) U of NM, Albuquerque, NM; 13) U of AL, Birmingham, AL.

The Family Investigation of Nephropathy and Diabetes is a multi-center study that includes African American (AA; N=1004), American Indian (AI; N=883), European American (EA; N=537), and Mexican American (MA; N=1634) families ascertained through a proband with diabetic nephropathy (DN). Association tests for type 2 diabetes (T2D) were performed utilizing 5548 SNPs in each population, which were originally genotyped for genome-wide linkage scans. The discrete trait option in the program Quantitative Transmission Disequilibrium Test was used. In MA, $p<0.001$ was observed for five SNPs (chr6, rs412735, p=0.0009; chr10, rs7899305, p=0.0009; chr13, rs1886040, p=0.0006; chr16, rs1389504, p=0.0007; chr18, rs680798, p=0.0008). The most significant p-value in EA was seen on chr18 (rs1075470, p=0.0012), in AA on chr10 (rs749694, p=0.0011), and in AI on chr3 (rs1495704, p=0.0011). In all, 21, 26, 34, and 49 SNPs showed $p<0.01$ in EA, AA, AI, and MA, respectively. Six SNPs overlapped with regions identified in linkage scans in the same populations. Furthermore, 15 of these SNPs were identified in previously published association studies. Candidate genes include insulin-like growth factor 2 mRNA-binding protein 2 (*IGF2BP2*, chr3q28) and stromal cell-derived factor 2-like (*SDF2L1*, chr22q11.2). The current study identified several SNPs associated with T2D in families ascertained for DN. Some of these associations replicate previous studies, while others require fine-mapping to determine their potential contribution to T2D susceptibility.

Development of a high-throughput linkage analysis system and application for the linkage analysis of familial multiple system atrophy (MSA). *Y. Fukuda[1], Y. Nakahara[1], H. Date[1], Y. Takahashi[1], J. Goto[1], K. Hara[2], M. Nishizawa[2], E. Nakamura[3], H. Adachi[3], S. Tsuji[1]* 1) Dept Neurology, Univ Tokyo, Tokyo, Japan; 2) Dept Neurology, Brain Research Institute, Niigata Univ, Niigata, Japan; 3) Dynacom Co., Ltd, Kanagawa, Japan.

High-throughput linkage analysis system dealing with enormous number of SNP data has been developed to enable rapid identification of the loci for disease-associated genes. In this system, SNP chip data can be directly imported and passed to pair-wise (mlink) or multipoint (allegro) linkage analysis programs. The system provides all parameter setting functions pre-included in the original programs and various marker-selecting functions such as HWE test. Furthermore, inter-marker distance can be flexibly chosen to adopt markers not in linkage disequilibrium. The current system can analyze the data of Affymetrix Genome-Wide Human SNP array 6.0 as well as Mapping 100K array and SNP array 5.0. Using this system we have conducted linkage analysis of multiple system atrophy (MSA) including seventeen individuals of six families genotyped by SNP array 6.0. All of the affected patients are sibling cases with more than one affected siblings. There are consanguineous marriages in the two families and affected patients are present in the upper generations in one family. Parametric multipoint analysis (autosomal recessive model) showed four loci with HLOD of 1.5 while nonparametric multipoint analysis showed six loci with NPL score of 2.0 and two loci of them were consistent with those found by parametric analysis. Highest HLOD and NPL of 2.22 and 2.63, respectively, were found in different genomic loci. These results raise the possibility of genetic heterogeneities of MSA, necessitating further accumulation of data to get more reliable results. Pair-wised linkage analysis employing 0.53 million SNPs was smoothly conducted by conventional PC within four hours. For multipoint analysis, process took less than 30minutes when inter-marker distances were set to 100kb. This system can be widely applied for linkage analysis using microarray-based SNP data and there one can expect high-throughput and reliable linkage analysis.

An integrated analysis workflow for high-throughput analysis of copy number variation for discovery and clinical diagnostics. *X. Gai, J. C. Perin, H. Xie, J. T. Glessner, S. F. A. Grant, H. Hakonarson, E. F. Rappaport, T. H. Shaikh, P. S. White* The Children's Hospital of Philadelphia, Philadelphia, PA.

Recent evidence suggests that genome copy number variations (CNVs) are associated with a substantial portion of inherited and acquired risk for various human diseases. The increasing availability of high-resolution genome surveillance platforms provides opportunity for rapidly assessing research and clinical samples for CNV content. To facilitate this process, we have developed a suite of software tools and resources (CHOPPY) that allows automated genome-wide CNV detection from genotyping data with high resolution and accuracy. A variety of array platforms are accommodated, including all recent Illumina and Affymetrix formats. CHOPPY is designed for high-throughput and large-scale copy number analysis using both signal intensity and allelic ratio values as input. CNVs as small as 2 SNPs can be reliably detected with a high experimental validation rate. The CHOPPY web interface is integrated with a local installation of the UCSC Genome Browser. Detected CNVs are stored in a relational database and presented either as a UCSC annotation track or in a tabular format for a given sample or cohort. The presentation layer also provides contextual genomic annotations for each CNV, including gene content, known disease loci, gene-based literature references, graphical and browsable genome representation, and prior CNV content from both the Database of Genomic Variants and the CHOP CNV Map of over 54,000 CNVs. This platform has been utilized for several disease-focused CNV surveys and has been implemented as the front-line system for assessing all clinical genetic samples received at the Hospital. In validation, CHOPPY independently identified pathogenic CNVs for 153 of 156 patients with known or suspected genomic disorders. CHOPPY represents a cohesive and convenient platform for detection, annotation, and assessment of the biological and clinical significance of human CNVs.

Incidence of vertebral fracture is associated with IL1 and IL6 polymorphisms in a large case-control sample selected from Study of Osteoporotic Fractures (SOF). *N. Aziz[1], H.-Y. Wang[1], K. Kornman[1], J. Zmuda[2], L.-Y. Liu[3], K. Stone[3]* 1) Interleukin Genetics, Waltham, MA; 2) Department of Epidemiology, University of Pittsburgh, Pittsburgh, PA; 3) Research Institute, California Pacific Medical Center, San Francisco, CA.

Vertebral fractures (VF) are a common clinical manifestation of osteoporosis which often lead to back pain, disabilities, kyphosis and subsequent fractures. Although the lifetime risk of developing a VF varies among women, relatively little is known about the genetic basis of this variability. We conducted a case-control study to test whether increased risk of VF are associated with certain alleles of the Interleukin1A (IL1A), Interleukin1B (IL1B), Interleukin1RN (IL1RN) and Interleukin6 (IL6) genes. The molecular basis of how some of these SNPs affect the IL1 gene activity have been previously investigated and may be related to the chronic hyper-inflammatory state thought to be an underlying causative factor in osteoporosis. 2527 Caucasian women (65 years) from the SOF cohort who reported never using estrogen replacement therapy were selected. Cases were defined by the presence of either prevalent VF (PVF) assessed at baseline or an incident VF (IVF) after an average of 3.7 years of follow-up. Controls were randomly selected from participants without either PVF or IVF. Logistic regression models were used to test the association between SNPs and VF, after adjustment for age and BMI. SNPs within the IL1B(rs16944), IL1A(rs17561), IL1RN(rs419598) and IL6(rs1800795) genes were strongly associated [OR ranging from 1.39-4.64, ($p<0.05$)] with IVF and/or PVF. These genetic biomarkers could be useful in the medical management of osteoporosis by identifying individuals at high risk of VF, so that preventative therapies can be administered. In addition, these markers could also be utilized as a selection tool in enriching for patients predisposed for the incidence of VF in clinical trials of investigational drugs.

Proteomic analysis to identify candidate genes influencing high-density lipoprotein particle size in obese individuals. *L. A. Collins[1], S. P. Mirza[1], T. Mersha[1], L. Martin[2], A. H. Kissebah[1], M. Olivier[1]* 1) Medical College of Wisconsin, Milwaukee, WI; 2) Children's Hospital, Cincinnati, OH.

Obesity is associated with a significant risk for cardiovascular co-morbidities, primarily mediated by a preponderance of small, dense high-density lipoprotein (HDL_3) particles which exhibit potentially pro-atherogenic capabilities. HDL_3 particles in obese individuals appear to be functionally defective as they lack their potent anti-oxidative and anti-inflammatory properties. In our Metabolic Risk Complications of Obesity Genes (MRC-OB) study, we analyzed the lipoprotein particle profile in 532 individuals from our cohort, and identified a quantitative trait locus for HDL median particle diameter on human chromosome 12p13 (LOD = 3.15). The interval contains 144 annotated genes, none of which have a known role in lipid or lipoprotein metabolism.

We isolated HDL fractions from human serum samples of obese and lean sibling pairs from our cohort using non-denaturing fast protein liquid chromatography. Proteins were isolated from HDL particles by chloroform extraction and quantified using isotopic labeling and tandem mass spectrometry. Our analysis identified six proteins significantly altered in obese individuals with small median HDL diameter (APOA2, C3, C4A, GC, HP, TTR). Using Ingenuity Pathway and Gene Ontology Enrichment analyses, we identified five genes (C1S, VWF, C1RL, CD163, PTPN6) in the QTL interval on chromosome 12 correlated to the altered proteins. We analyzed 35 SNPs spanning the von Willebrand factor (VWF) gene and detected significant association with HDL median particle diameter for four SNPs ($p = 0.004$-0.045). One of these SNPs, rs216321, alters the amino acid sequence (Arg852Gln) of the protein. Further studies will determine the functional mechanism by which VWF influences HDL particle size in obese individuals.

GSNO Reductase and $_2$ Adrenergic Receptor Gene-gene Interaction: Bronchodilator Responsiveness to Albuterol. *S. Choudhry[1], Z. Yang[2], L. Liu[1], C. Eng[1], S. O. Kim[3], G. Kumar[1], S. Thyne[1], R. Chapela[4], K. Meade[5], H. G. Watson[6], M. LeNoir[7], J. R. Rodriguez-Santana[8], W. Rodriguez-Cintron[9], P. C. Avila[10], J. S. Stamler[2], E. G. Burchard[1], L. G. Que[2]* 1) University of California, San Francisco; 2) Duke University, Durham; 3) Taeyeon Medical CO. LTD, Republic of Korea; 4) INER, Mexico; 5) CHORI, Oakland; 6) The James A. Watson Wellness Center, Oakland; 7) Bay Area Pediatrics, Oakland; 8) CSP, San Juan; 9) Veterans Caribbean Health Care System, San Juan; 10) Northwestern University, Chicago.

Short-acting inhaled $_2$-agonists such as albuterol are the mainstay of asthma treatment worldwide. There is significant variation in bronchodilator responsiveness to albuterol not only between individuals but also across racial/ethnic groups. $_2$ adrenergic receptor ($_2$AR) is the target for $_2$-agonist drugs used for bronchodilation in asthma. Recently, S-nitrosoglutathione reductase (GSNOR) has been shown to regulate endogenous bronchodilator tone. We hypothesized that variation in response to albuterol could be partly due to pharmacogenetic interactions between GSNOR and $_2$AR genes. We performed family based and cross-sectional analyses to test for association between GSNOR gene variants and asthma and related phenotypes in 684 Puerto Rican and Mexican families with asthma and 440 African American asthma cases and controls. We also tested these subjects for pharmacogenetic interaction between GSNOR and $_2$AR gene variants and response to albuterol. To test the potential effect of the GSNOR gene variants, mRNA secondary structure analyses and cell transfection experiments were performed. Among Puerto Ricans, several GSNOR SNPs and a haplotype were significantly associated with asthma and responsiveness to albuterol ($p = 0.04$ to 0.008). The GSNOR 3UTR risk haplotype affects mRNA secondary structure and luciferase reporter expression. Furthermore, GSNOR-$_2$AR interaction analysis provided strong evidence of association with responsiveness to albuterol in both Mexican and Puerto Rican (Latino) asthmatics ($p_{combined} = 0.001$). We conclude that genotyping of GSNOR and $_2$AR genes may be a useful approach for identifying subjects that might benefit from adjuvant therapy for refractory asthma.

Sixteen-year Review of the Results of Chromosomal Analysis at the Cytogenetics Laboratory of the Institute of Human Genetics, National Institutes of Health Philippines. *C. D. Padilla[1,2], N. S. Cadag[1], M. D. Chiong[1,2], E. M. C. Cutiongco[1,2]* 1) Institute of Human Genetics, National Institutes of Health Philippines, Manila, Philippines; 2) Department of Pediatrics, College of Medicine-Philippine General Hospital, University of the Philippines Manila.

The field of human cytogenetics is an increasingly important area of medicine as it has helped elucidate the etiology of many congenital malformation/mental retardation syndromes and various chromosomal abnormalities which constitute a substantial proportion of human morbidity and mortality. This paper presents a 16- year review of the results of the chromosomal analysis done at the Cytogenetics Laboratory of the Medical Genetics Unit, College of Medicine and the Institute of Human Genetics, National Institutes of Health, University of the Philippines Manila. Results of the chromosomal analysis done on peripheral blood samples from the period of July 1991-December 2007 were retrospectively reviewed. Among the clinical indications for cytogenetic analysis were congenital anomalies, mental retardation, disorders of sex differentiation, infertility or recurrent miscarriages, hematologic malignancies and other cancers, prenatal diagnosis and exposure to radiation and toxic chemicals. Chromosomal analysis was limited to G-banding techniques and evaluation of gross numerical and structural appearance of the chromosomes. Out of 10,857 peripheral blood samples that were analyzed, 30.3% (3,293 of 10,857) had abnormal results. The major type of chromosomal aberrations seen were numerical chromosome abnormalities which accounted for 67% of the cases, of which Trisomy 21 was the most predominant of the aneuploidies reported. Sex chromosome abnormalities were the second most common chromosomal abnormalities seen with Turner Syndrome accounting for 82.6% of the cases. Different types of structural chromosome abnormalities were observed in 11.1% of all chromosomal abnormalities.

The GATA2 transcription factor gene - promoter haplotypes and preliminary association with neuropsychiatric phenotypes. *M. A. Kennedy[1], M. V. Bland[1], R. L. Roberts[1], P. C. McHugh[1], K. J. Light[2], A. L. Miller[1], P. R. Joyce[2]* 1) Department of Pathology, University of Otago, Christchurch, New Zealand; 2) Department of Psychological Medicine, University of Otago, Christchurch, P.O. Box 4345, Christchurch, New Zealand.

The central serotonergic (5-HT) neurotransmitter system is consistently implicated in mood disorders and anxiety, as well as behavioural phenotypes such as neuroticism or harm avoidance. We are exploring the hypothesis that genetic variability which may impact on the development and architecture of the 5-HT system may also be a contributing factor to relevant neuropsychiatric phenotypes. The transcription factor GATA2 plays a key role in controlling 5-HT differentiation of neuronal precursor cells. We surveyed the coding, splice junctions, 5UTR and core promoter regions of the GATA2 gene for common variants. Eight common variants were discovered in the promoter region including a 4bp indel. These variants formed six distinct haplotypes, four of which were found to be relatively common in the population (5%). Reporter gene assays in COS-7 and the rat 5-HT cell line RN46A demonstrated marked differences in basal expression between the common haplotypes. We then carried out a genetic association study in a large family-based study of depression, for whom we also had personality measures (TCI). PCR and SNaPshot assays were used for genotyping. One haplotype (containing the 4bp deletion) was significantly associated with risk of major depression (chi-square = 6.721, P = 0.0096) with a three to four-fold (95% CI 2.24 - 5.77) increased relative risk. In addition, this haplotype was associated with higher harm avoidance, when gender was included as a modifier (chi-square= 8.497, P= 0.004), with a 14% (95% CI 11% - 16%) increase in score compared to all other common haplotypes. Identification of these GATA2 promoter variants and demonstration of functional effects suggest that this gene should be considered a candidate in phenotypes linked to 5-HT dysfunction. Our preliminary association studies suggest a possible link to risk of depression and harm avoidance, although these findings require replication.

INADL and MYT1L are associated with risk for anencephaly. *C. F. Potocky[1], N. Ellis[1], A. Trott[1], C. S. Haynes[1], D. G. Siegel[1], H. Cope[1], K. Soldano[1], D. S. Stamm[2], A. Dellinger[1], P. Xu[1], T. M. George[3], S. G. Gregory[1], A. E. Ashley-Koch[1]* 1) Center for Human Genetics, Duke Medical Center, Durham, NC; 2) University of California, Davis School of Medicine, Sacramento, CA; 3) Dell Children's Medical Center of Central Texas, Austin, TX.

We previously identified INADL and MYT1L as novel candidate genes for anencephaly by a genome wide association study (GWAS) (Stamm et al., 2007) and differential expression in fetal neuronal longSAGE libraries (Xu et al., 2007). InaDL (inactivation no afterpotential D-like) helps control epithelial migration (Shin et al. 2007) and is critical in establishing cell polarity (Michel et al. 2005; Li et al. 2004). Myt1L (myelin transcription factor 1-like) is a zinc finger DNA binding protein. Both Myt1 and Myt1L are found in neurons at early stages of differentiation (Bellefroid et al. 1996; Jiang et al 1996; Kim et al. 1997; Weiner and Chun 1997; Yee and Yu 1998). Here we report fine mapping of these genes in an expanded dataset. The initial GWAS dataset contained 45 anencephaly families and 5 families with other cranial neural tube defects (NTDs). The follow-up data set was comprised of 86 anencephalic families and 22 families with other cranial NTDs, inclusive of the GWAS families. Three affection models were considered for analysis: anencephaly only, all cranial NTDs, and any NTD regardless of lesion location. These phenotypes were analyzed for association with 26 SNPs over a 400kb region including INADL and 26 SNPs over a 550kb region including MYT1L using APL and hAPL. Two nonsynonymous coding SNPs within INADL were significantly associated (rs1056513 and rs1134767, APL $p<0.01$ for both SNPs in all 3 models). One of these coding SNPs, rs1134767, creates a highly significant haplotype with rs6697273, the INADL SNP originally identified in the GWAS (hAPL $p<0.001$ for all 3 models). Association was also confirmed in MYT1L with rs12470297 showing significant association, particularly in the anencephaly subset (APL $p=0.0007$). In conclusion, fine mapping confirmed INADL and MYT1L are associated with risk for NTDs and identified two nonsynonymous coding SNPs with significant association to cranial NTDs.

Comparison of genome-wide recombination intensity from pedigree/linkage data to population recombination rates estimated from SNP genotype data in the European-American population. *X. Wang[1], J. Li[2], S. Buyske[3], T. Matise[3], A. Clark[1]* 1) Cornell University, Ithaca, NY; 2) University of Missouri-Kansas City, Kansas City, MO; 3) Rutgers University, Piscataway, NJ.

We estimated genome-wide local recombination intensity by local regression on the most comprehensive Rutgers human linkage map with 28,121 polymorphic markers and more than 1000 informative meioses. The bandwidth, local polynomial degree and weight function parameters were optimized by minimizing the local likelihood among a grid of parameters for each chromosome arm. For the genome-wide population recombination rate, we used the Illumina 317k SNP data of the 1000 control samples of the PARC study. Through a large number of simulations, we found that the widely used composite likelihood method for estimating population recombination rate (implemented in LDhat program) is sensitive to population demographic history, MAF filtering and tagging SNP selection. The population recombination rate was estimated in sliding windows spanning the genome using a window size of 40kb and an increment of 20kb, correcting for demographic history, MAF ascertainment bias, and tagging SNP selection effects based on the simulation studies. The population recombination rate was then compared with the recombination intensity from deCODE map and the Rutgers genetic map, with the matched band width parameter. The gross impression is a good correspondence between the two maps, however there are locally discrepant regions. One source for the discrepancies could be the inaccuracy of estimation caused by locally low marker density. Because the population recombination rate is defined as $=4N_e r$, where N_e is the effective population size and r is the recombination rate, natural selection could reduce N_e locally and distort the estimate of . In addition, recombination intensity represents a snapshot of the recombination rate at the present time, whereas the population recombination rate integrates the recombination rate over recent ancestral history of the sample. From the comparison of these recombination estimates, we can also make much more accurate inference about local N_e and genome average N_e than other existing methods.

Genome Wide Association Studies in Prospective, Community-Based Cohorts: the CHARGE (Cohorts for Heart and Aging Research in Genomic Epidemiology) Consortium. *C. J. O'Donnell[1,6], E. Boerwinkle[2,6], V. Gudnason[3,6], A. Hofman[4,6], B. M. Psaty[5,6]* 1) NHLBI's Framingham Heart Study, Framingham MA; 2) University of Texas Health Science Center, Houston TX; 3) University of Iceland, Reykjavik, Iceland and National Institutes of Aging, Bethesda MD; 4) Erasmus Medical Center, Rotterdam, Thethe Netherlands; 5) University of Washington, Seattle WA; 6) On behalf of the CHARGE Consortium.

Introduction: Recently, meta-analysis of findings from multiple genome-wide association studies (GWAS) have been used to discover many novel genetic variants with strong evidence for replication for common diseases. GWAS in large, prospective community-based cohorts offer a unique opportunity to discover genetic determinants of multiple well-characterized disease traits, using strategies such as longitudinal analyses for quantitative traits and time-dependent analyses for outcome measures. Methods and Results: We formed an international consortium of prospective, community based cohorts with >38,000 white participants: the Age, Gene/Environment Susceptibility Study, Atherosclerosis Risk in Community Study, Cardiovascular Health Study, Framingham Heart Study and Rotterdam Study. Hundreds of similarly measured qualitative and quantitative phenotypes in persons free of disease are used, including cardiovascular disease (CVD) risk factors, blood biomarkers, electrocardiography, and coronary artery atherosclerosis; as well as aging and CVD endpoints, such as myocardial infarction and stroke, occurring years after collection of DNA. Over twenty CHARGE Phenotype Working Groups adopted principles of collaboration/authorship and statistical analysis strategies to design and analyze GWAS. Genotyping platforms differed among the studies, so imputation methods were used to create a common set of ~2.5 million HapMap SNPs (Release 22, Build 26, CEU) for meta-analyses, scheduled to proceed during the summer and fall of 2008. Conclusion: The international CHARGE Consortium will provide GWAS data from >38,000 participants in five cohorts for multiple CVD and aging phenotypes. Genetic variants with strong evidence of replication in CHARGE will warrant further follow up.

Comparison of family-based association analysis methods for SNP-haplotypes in nuclear families and extended kindred. *M. Govil, T. Goldstein McHenry, M. L. Marazita* Center for Craniofacial and Dental Genetics, University of Pittsburgh, PA.

Many methods now exist for conducting family-based haplotype association analyses of SNP genotypic data, each with a different software package for implementation of the method. The issue that plagues most users is which of the methods/software would be most appropriate for their study design and desired analyses. If more than one method/software can be used for the desired analyses, but differences are seen in the results, which result should be chosen? In our analyses of the genetics of craniofacial birth defects in extended kindred from multiple populations, we found orders-of-magnitude differences in the results from HBAT and UNPHASED -- two commonly used family-based haplotype association analysis methods -- and decided to investigate the causes behind these differences. We selected three methods/software for comparison: the haplotype version of FBAT (HBAT), UNPHASED, and MENDEL. Both HBAT and UNPHASED compute a conditional likelihood, although via different algorithms: EM for HBAT and either DFP or Nelder-Mead for UNPHASED. HBAT computes an empirical estimate of the variance which allows one to account for correlation between nuclear sub-families. Using allele combining options along with the gamete competition model allows computation of haplotype association via MENDEL. We selected extended kindred, nuclear families and trios as the data for our comparisons, with moving windows of 2-5 SNPs across 26 total SNPs. Our tests show a difference between results from HBAT and UNPHASED even for the trios. The HBAT results were more comparable to MENDEL than were the UNPHASED results. While further testing and simulations are necessary to fully understand the reasons behind the observed differences in results, clearly a cautionary note is necessary when a genetic study design includes haplotype association analyses. NIH grants # P50-DE016215, R01-DE DE016148, U01- DE018903.

Whole blood genome-wide gene expression in the KORA population. *D. D. Mehta[1], K. Heim[1], T. Illig[2], H. E. Wichmann[2,4], T. Meitinger[1,3], H. Prokisch[1,3]* 1) Institut für Humangenetik Helmholtz Zentrum München, Deutsches Forschungszentrum für Gesundheit und Umwelt Ingolstädter Landstr. 1 85764 Neuherberg Germany; 2) Institut für Epidemiologie Helmholtz Zentrum München, Deutsches Forschungszentrum für Gesundheit und Umwelt Ingolstädter Landstr. 1 85764 Neuherberg Germany; 3) Institut für Humangenetik Technische Universität Klinikum Rechts der Isar Trogerstr. 32 81675 München Germany; 4) Institute of Medical Informatics,Biometry and Epidemiology, LMU, Munich, Germany.

We have setup a large resource consisting of 350 whole blood genome-wide gene expression data and 300 LCLs from the KORA population. Normal variation in gene expression was assessed using the Illumina Human Ref6v2 whole genome microarray. Our large gene expression resource can be useful in several aspects 1) Several significant age-related genes and gender-specific gene signatures were identified. Using the PAM algorithm, it was possible to predict the gender with an accuracy of 95%. Numerous gene expression profiles correlated with metabolites such as high density lipoproteins. 2) Using KORA Affymetrix 500k genotypes, we performed a genome-wide association study to compare blood eQTLs to published LCL eQTLs. 3) Expression data can be used to test for functional SNPs. In this context, a recent genome wide association study using the KORA population found the most significant SNPs associated with urate levels mapped within an uncharacterized carrier gene SLC2A9. We carried out an isoform-specific gene expression analysis, revealing a significant association between SLC2A9 isoform 2 expression and urate levels (Doering et al, Nature Genetics 2008). 4) We used our expression resource in a candidate approach to test for previously described associations at the transcript level. Our data adds resolution to genome-wide association studies and extends the current limited knowledge of whole blood genome-wide gene expression in the general population. References SLC2A9 influences uric acid concentrations with pronounced sex-specific effects : Angela Doring, Christian Gieger, Divya Mehta, Henning Gohlke, Holger Prokisch et al, Nature Genetics 2008.

Lipin-1 Genetic Variation May Influence Liver Function and Inflammation in Hispanic Americans. *M. O. Goodarzi[1], X. Guo[1], Y. I. Chen[1], B. Fang[1], A. Xiang[2], K. D. Taylor[1], T. Buchanan[2], L. J. Raffel[1], J. I. Rotter[1]* 1) Cedars-Sinai Med Ctr, Los Angeles, CA; 2) USC, Los Angeles, CA.

Lipin-1 influences adipogenesis and insulin sensitivity in adipose tissue and the liver. It was initially identified as the locus responsible for the fatty liver dystrophy (*fld*) mouse, which is characterized by absence of adipose tissue depots throughout the body, transient neonatal fatty liver, and peripheral neuropathy. As a key factor in adipogenesis, human adipose lipin-1 mRNA levels are inversely correlated with whole-body insulin resistance, suggesting that by moving fat into adipose tissue, lipin-1 maintains insulin sensitivity by preventing fatty infiltration of liver and skeletal muscle. Furthermore, lipin-1 mRNA levels have been found to be inversely correlated with adipose tissue expression of inflammatory cytokines. Thus, we performed a pilot study to determine whether variants in the gene for lipin-1 (LPIN1) were associated with the liver enzyme gamma glutamyl transferase (GGT, a marker for fatty liver) or inflammatory markers (C-reactive protein, serum tumor necrosis factor (TNF) receptor 1 (TNFR1) and receptor 2 (TNFR2)). The study cohort consisted of 618 non-diabetic offspring from 160 Hispanic-American families ascertained through a proband with hypertension. Two SNPs on opposite ends of the LPIN1 gene were genotyped, haplotypes constructed, and tested for association using generalized estimating equations (GEE1) to account for familial correlation, adjusting for age, sex, and BMI. Haplotype 1 (most common haplotype) was associated with an increase in GGT (haplotype carriers 31.71.1 vs non-carriers 29.85.1 U/L, p=0.026). SNP rs11524 was associated with decreased TNFR1 (1.770.035 vs 1.830.020 ng/mL, dominant model, p=0.029). Haplotype 2, which carries rs11524, exhibited the same association. The functional significance of these variants is unclear and will require fine-mapping; however, computational modeling suggests that rs11524 alters an exonic splicing silencer sequence (Ong KL, et al. Am J Hypertens 2008;21:539-45). Consistent with predictions based on the biology of lipin-1, variants in the LPIN1 gene may modulate liver function and inflammation.

Haplotype Differentiation in Chromosomes 21 and 22 among the Korean and International HapMap populations. *C.-H. Yi[1,2], M.-H. Kim[1], E. J. Kang[1], H. G. Kim[1], H. J. Kang[1], S. K. Lee[1], J.-A. Pyun[1], S. S. Won[1], K.-Y. Kim[1], J.-G. Kim[1], K.-D. Lee[1], H.-J. Song[1], A.-R. Oh[1], J.-H. Chung[3], K. B. Kwack[1]* 1) Medical Genomics Laboratory, CHA Research Institute, Pochon CHA University, Seongnam, Kyunggido 463-836, Republic of Korea tel:+82-31-725-8376; 2) Institute of Environment and Ecology, 1, Anamdong 5-ga, Sungbuk-gu, Seoul136-701, Republic of Korea; 3) Kohwang Medical Institute, School of Medicine, Kyung Hee University, Seoul 130-701, Republic of Korea.

A Korean sample (KOR) has been genotyped so as to perform systematic comparison with Japanese (JPT), Northern Chinese (CHB), Caucasians (CEU), and Africans (YRI) sampled for the International HapMap Project. As the three Northeast-Asian populations share a considerable human genetic ancestry, the genetic similarity of the Koreans either to Japanese or to Chinese varied between both chromosomes, a sign of fine-scale genetic differentiation and different population history on different chromosomal regions. With the same single nucleotide polymorphism (SNP) markers (1370 and 1866 SNPs on chromosome 21 and 22, respectively) in Illumina Human 1, 153 and 230 haplotype blocks were delimited in Koreans for chromosomes 21 and 22, respectively. The total numbers of haplotype blocks in Koreans, Japanese and Northern Chinese varied between the smallest from Africans and the greatest from Caucasians. Haplotype patterns in combined samples like KOR+JPT, KOR+CHB, KOR+JPT+CHB showed variable numbers of haplotype blocks. Twenty-six chromosomal regions containing long-range haplotypes in reference to the Korean population were selected and analyzed further. Population-wise long-range haplotype blocks could be mapped out. The typically expected pattern, the Northeast-Asian triplet (KOR-JPT-CHB) in contrast to Caucasians (CEU) and Africans (YRI), came out slightly more than half of the cases compared, while variable pairing and tripling patterns had been observed. The fine-scale differentiation among KOR, JPT and CHB backs up the needs for systematic sampling and genotyping of a greater number of ethnic groups in the Northeast Asia.

Duplications in the 17p13.3 Miller-Dieker syndrome region: Increased expression of *LIS1* affects human and mouse brain development. *W. Bi[1], T. Sapir[3], O. A. Shchelochkov[1,2], F. Zhang[1], M. Withers[1], J. V. Hunter[2], T. Levy[3], V. Shinder[4], D. A. Peiffer[5], K. L. Gunderson[5], X. Lu[1], T. Sahoo[1], Y. Yanagawa[6], A. L. Beaudet[1,2], S. W. Cheung[1], S. Martinez[7], J. R. Lupski[1,2], O. Reiner[3]* 1) Dept Molec & Human Genetics, Baylor Col Medicine, Houston, TX; 2) Texas Children's Hospital, Houston, TX; 3) Dept. Molecular Genetics, The Weizmann Institute of Science, Rehovot, Israel; 4) Dept. Chemical Research Support, The Weizmann Institute of Science, Rehovot, Israel; 5) Illumina, Inc., San Diego, CA; 6) Dept. of Genetic and Behavioral Neuroscience, Gunma University Graduate School of Medicine, Maebashi, Japan; 7) Instituto de Neurociencias, UMH-CSIC, San Juan de Alicante, Alicante, Spain.

Deletions of the *LIS1* gene in human chromosome 17p13.3 result in isolated lissencephaly sequence and the extended deletions including the *14-3-3* gene cause Miller-Dieker syndrome (MDS). By array CGH we identified seven unrelated cases with duplication ranging from 240 kb to 3.6 Mb in 17p13.3 involving the *LIS1* and/or the *14-3-3* genes. Duplications in three cases are complex and may represent the autosomal rearrangements generated by the DNA replication FoSTeS mechanism. Real-time RT-PCR showed that the expression levels of the *LIS1*, *14-3-3*, and *CRK* genes were increased when these genes are duplicated. Using a forward genomics approach we characterized the clinical consequences of duplications. Increased *LIS1* dosage causes smaller brains, mild brain structural abnormalities, moderate to severe developmental delay, and failure to thrive. Duplication of *14-3-3* and surrounding genes increases the risk for macrosomia, mild developmental delay, pervasive developmental disorder, and results in shared facial dysmorphologies. Transgenic mice conditionally over-expressing LIS1 in the developing brain exhibited a decrease in brain size, an increase in apoptotic cells, and a distorted cellular organization in the ventricular zone including reduced cellular polarity. Collectively, our results show that an increase in LIS1 expression in the developing brain results in smaller brains and neuronal migration abnormalities in mice and human patients.

Alpha sarcoglycan gene is down-regulated by Sox9 in a TGF -dependent process during myogenesis. *J. M. Hernández Hernández[1], P. Delgado Olguín[1,2], V. Aguillón[1], M. Furlan Magaril[2], F. Recillas Targa[2], R. M. Coral Vázquez[1]* 1) UIM en Genética Humana,CMNS XXI-IMSS. Av. Cuauhtémoc 330 México DF 06725; 2) Genética Molecular, Instituto de Fisiología Celular UNAM, México DF.

The sarcoglycan sarcospan complex is composed of the transmembranal proteins , , , , SG's as well as sspn. Mutations in , , , SG cause autosomal recessive limb girdle muscular dystrophies. SG is expressed exclusively in striated muscle during myogenesis, which can be explained by multiple cis regulatory elements on their promoter region. Recently, our group has demonstrated the interaction of MyoD with the transcription factors TFIIB and TFIID in the SG promoter region to upregulate the expression of this gene. In order to know the mechanisms involved in their negative regulation, we analyzed the sox9 participation through analysis of a reporter activity driven by SG promoter in C2C12 cells cotransfected with sox9. Interestingly, the promoter was down regulated 80% in myoblasts, but not in myotubes where the endogenous sox9 protein is undetectable. We analyzed the endogenous SG mRNA level in a stable line that overexpress Sox9. The transcript was reduced 60% with respect to wild type cells, as well as the protein. To analyze the functionality of the sox elements, we carried on EMSA assays by using both nuclear extracts of myoblasts and transcription-translation extracts of sox9, and we identified complexes which were super-shifted with a specific antibody against sox9. ChIP assays showed, in myoblasts but not in myotubes, the interaction of both sox9 and p-smad3 with the three elements of the promoter. These data suggest a model where TGF modulates the repression effect of sox9 and smad3 on the SG promoter. In agreement with this, TGF increased dramatically the promoter repression induced by these transcription factors. Interestingly, the activity was recovered by the addition of a specific inhibitor of the TRI. Our results show that the repression exerted by Sox9 and smad3 on the promoter is sensible to TGF . This is the first demonstration that sox9 can directly bind to some specific DNA elements to negative regulate a muscle specific gene during myogenesis.

SNP based copy number microarrays provide cues to UPD, and recessive allele risk due to inbreeding. *P. Papenhausen, J. Tepperberg, I. Gadi* Dept Cytogenetics, Labcorp America, Res Triangle Park, NC.

SNP based chromosome microarrays can provide an extremely high density whole genome analysis of copy number variation in the clinical analysis of blood from children with developmental delay. We have studied about 2,000 cases this year using both the Affymetix 500k and 1.8 million SNP/copy number chips. The allele differentiation that the array provides allows designation of the relative distribution of homozygosity (HZ)(or loss of heterozygosity) thoughout the genome, in addition to copy number. We expected that long contiguous stretches of HZ(LCSHZ) >25Mb in a single chromosome would correlate with UPD based on the almost 2/3 of our reported 35 cases of UPD that showed regions of both hetero and iso UPD using limited numbers of microsatellites. The added significance of HZ determinations was not fully appreciated until the following cases were noted (it is important to understand that contiguous HZ over 5Mb is rarely observed in these studies): a 3yo showed 27 LCSHZ's (>10Mb) on 20 chrs, a 5yo had 8 LCSHZ's on 7 chrs, a 2yo had 10 LCSHZ on 8 chrs, a 12yo showed 12 LCSHZ's on 11 chrs, a 10yo had 11 LCSHZ's on 11 chrs and a 32yo had 9 LCSHZ's on 7 chrs. All cases proved to represent consanguinity with the first case a product of a brother-sister pairing. Additional probands from second cousin pairings showed smaller blocks with fewer chromosomes involved, as what might be expected from multi-generation recombination and dilution of consanguinous chromosomes. A separate pattern of cases showed greatly increased homozygosity, but without contiguous stretches. These cases appeared to represent geographical or ethnic isolates with limited outbreeding. It will be important to determine the LCSHZ boundaries for levels of consanguinity and a reasonable means of reporting these findings which have implications for recessive allele risk. Patterns of homozygosity with possible threshold ranges and UPD examples will be presented. Although LCSHZ in a single chromosome correlates with UPD, confirmation in an imprinted chromosome through paternal microsatellite exclusion or direct methylation specific testing is highly recommended.

Clinical testing for mutations in the *PAX2* gene and diagnosis of renal coloboma syndrome. *M. Bower[1], C. Xu[2], R. Peterson[2], L. Schimmenti[1], X. Wang[1,2]* 1) Genetics and Metabolism, University of Minnesota Medical Center, Fairview, Minneapolis, MN; 2) Laboratory Medicine and Pathology, University of Minnesota Medical Center, Fairview, Minneapolis, MN.

Renal coloboma syndrome (RCS) is an autosomal dominant disorder characterized by ocular and renal malformations. Mutations in the paired-box gene, *PAX2*, have been associated with RCS. Our laboratory developed the first clinical testing service for *PAX2* mutations in 2007. Testing involves bi-directional sequencing of all 12 coding exons of the *PAX2* gene and adjoining intronic sequences. To date, we have completed 30 cases representing 26 unique families. Mutations in the *PAX2* gene were identified in 7/26 probands. Mutations were not identified in any of the 11 probands with atypical findings, such as iris coloboma. Consistent with prior observations, the majority (6/7) of the mutations identified in this sample were frameshift mutations that would be expected to result in a truncated PAX2 protein. Three of the mutations identified in our laboratory are novel mutations (c.250 G>A, c.836delG and c.894delTinsGC). Additional findings in mutation positive individuals included short stature, elevated pancreatic amylase, and hearing loss. One mutation positive proband with renal insufficiency and optic nerve colobomas had a pregnancy with a prenatal diagnosis of bilateral renal agenesis. Of the seven mutation positive cases, five occurred in the absence of any family history, suggestive of a de novo origin for the mutation. In one case, the proband had an affected parent and multiple affected siblings. In the remaining case, the familial mutation was present in two affected siblings and absent in their father, who had bilateral abnormalities of the optic nerve. This latter case suggested the possibility of somatic mosaicism. In summary, mutations were found in approximately half of cases with classic findings for RCS. The majority of *PAX2* mutations appear to be de novo. There is evidence that mosaicism, both germline and somatic, may not be uncommon in RCS. The possibility of somatic mosaicism should be strongly considered in mutation negative cases with suggestive clinical findings.

Genetic counselors in Japan: A five-year experience. *C. Tamura* Genetic Counseling Graduate Program, Ochanomizu University, Tokyo, Japan.

Over the last several years, there has been some change in genetic counseling system in Japan. Genetic counseling has been conducted for more than thirty years by physicians. However, recently, some graduate programs have established to train non-MD genetic counselors. Japanese Board of Genetic Counseling was established in 2005 as the joint effort of two genetics societies, and certification examination was started following the accreditation of genetic counseling training programs. Until 2008, eight graduate programs have been accredited, and seventeen people were certified as genetic counselors; twelve were graduates from Japanese training programs and other five were people who had had equivalent training in some different ways. There have been some issues with these certified genetic counselors, which needs to be solved for the future. One of the critical problems is employment. It has been difficult for genetic counselors to find a job and the income is extremely low. The government and genetics societies have published some guidelines which stated that one of the major roles of clinical geneticists is genetic counseling provision, and so, the role of genetic counselors is not clear, as they tend to play an assistant role. Therefore, it has been difficult to determine the educational goal to train genetic counselors. Furthermore, it has been believed that there is a Japanese specific genetic counseling model, and this has caused confusion among genetic counselors. In Japan, prenatal testing has not been recommended, since termination of pregnancy because of fetal problems is theoretically illegal, though it is practically available under parents economical reasons. Clinical geneticists believe that it is better to for them to play a gate-keeping role for prenatal testing, considering whether the termination is permitted or not. Thus, they often try to convince women not to undergo prenatal testing which may be followed by termination, and this has become the Japanese gate-keeping model of genetic counseling. This model has tended to apply to other settings, including predictive testing, etc. In the future, non-directiveness in genetic counseling has to be discussed to appropriately train genetic counselors.

Technology as an Educational Enhancement Tool in the Fields of Human and Microbial Genetics: Impacting Students from High School through the Undergraduate Years. *T. N. Turley-Stoulig, T. Tinney* Department of Biological Sciences, Southeastern Louisiana University, Hammond, LA.

Technological advances in human genetics have presented educators with a wealth of tools to engage students when teaching and discussing complex and intriguing concepts and these tools are continually being developed and enhanced. We must stay abreast of this technology to ensure that students receive the most engaging educational experience possible. Also, as the field of genetics advances at a rapid pace, educators must make a continuous effort to familiarize themselves with new discoveries and information that may lead to revision of previous ideas and expose students to those discoveries. In connection with these advances in human genetics, the purpose of this study is to aid in the need for well-trained biologists/geneticists who are substantially exposed to these advances and to expose the public to concepts of genetics to aid them in making informed decisions regarding their future health and that of society as it relates to genetics and the advances being made. We used various tools to enhance the educational experience for students in the fields of microbial and human genetics from high school level to undergraduate level. These tools included interactive presentation, molecular genetics animation, database resources and genetics laboratory materials. At the high school level, our results showed that students became more engaged in their biology course and indicated a stronger interest in entering the biological sciences in the future. They also felt better prepared to approach, understand and make use of published news material relating to genetics. Teachers became more eager to investigate use of the tools for themselves in their classroom and more interested in providing students with material that connecting students' learning with real-life application. At the undergraduate level, our results showed that students felt better prepared when the tools were used and were more interested and often awed by the subject. We conclude that these tools improve the educational experience and we plan to extend this study to evaluate the effectiveness of these tools further.

Decreased antioxidant enzyme activity and mitochondrial DNA copy number in cellular models of Machado-Joseph disease. *M. Hsieh[1,2], Y. C. Yu[1], C. L. Kuo[3], W. L. Cheng[3], C. S. Liu[3,4,5]* 1) Dept Life Sci, Tunghai Univ, Taichung; 2) Life Science Research Center, Tunghai University, Taichung; 3) Vascular and Genomic Research Center, Changhua Christian Hospital, Changhua; 4) Department of Neurology, Changhua Christian Hospital, Changhua; 5) Department of Neurology, Chung Shan Medical University and Chung Shan Medical University Hospital, Taichung, Taiwan.

Machado-Joseph disease (MJD) is an autosomal dominant spinocerebellar degeneration caused by an unstable CAG trinucleotide repeat expansion in MJD gene. Because of the late on-set of the disease, we hypothesize that the accumulated oxidative stress or/and defective antioxidant enzyme ability may be contributory factors for the pathogenesis of MJD. In this study, we utilized SK-N-SH and COS-7 cells stably transfected with full-length MJD with 78 polyglutamine repeats to examine any alterations in antioxidant activities. We demonstrated a significant reduction in the ratio of GSH/GSSG and total glutathione content (GSH + (2XGSSG)) in mutant MJD cells as compared with the normal cells under normal or stressful conditions. We also showed that both SK-N-SH-MJD78 and COS-7-MJD78 - GFP cell lines have the lower levels of catalase, glutathione reductase and superoxide dismutase when compared to the wild-type cell lines under normal growth condition. In addition, it is known that when cells are under oxidative stress, the mitochondrial DNA is prone to damage. Our results demonstrated that mitochondrial DNA copy numbers in both mutant cells and MJD patients samples are decreased when compared to the normal controls. Furthermore, mitochondrial DNA deletion contents in MJD patients are significantly increased when compared to those from normal individuals. Taken together, mutant ataxin-3 might influence the activity of enzymatic components to remove O2- and H2O2 efficiently, and promote mitochondrial DNA mutation or deletion, which leads to dysfunction of mitochondria. Therefore, we suggest that the cell damage caused by greater oxidative stress in SCA3 mutant cells plays an important role, at least in part, in the disease progression.

The Impact of Lipid-related Gene in Memory. *H. Hsieh[1,2], Y. Yan[3], C. Liu[2,3], C. Lai.[2,3]* 1) Department of Psychiatry, Yuli Veterans Hospital, Yuli Town, Hualian County, Taiwan; 2) Graduate Institute of Neurological Sciences, Kaohsiung Medical University; 3) Department of Neurology, Kaohsiung Medical University Hospital.

Background The brain is the most cholesterol-rich organ in the human body. The metabolic syndrome, a clustering of several commonly disorders that include hyperlipidemia, may be a risk factor for memory decline in the elderly. Apolipoprotein E (Apo E) is a lipoprotein that transports cholesterol and other lipids. The Cyp46 enzyme is a member of the cytochrome P450 family of proteins. It regulates the elimination of excess cholesterol by adding a hydroxyl group to cholesterol. Previous studies have shown that high serum total cholesterol level is a risk factor for dementia, and may be as well as the clinical hallmark for progressive memory impairment. The aim of this study was to clarify the role of lipid-related gene (APOE, CYP46) in memory performance in middle-aged and elderly adults without dementia. Methods Using cross-section design, 209 cognitively intact participants were recruited. The mean age (SD) was 67.87 (6.80) years, the mean duration of education was 11.07 (3.91) years, and 49.76% were women. Memory and learning function were measured by logical memory (I) (II), world list (I) (II) and spatial span of the Wechsler Memory Scale-III (WMS-III). 3 single nucleotide polymorphisms at the APOE gene and 7 tagging single nucleotide polymorphisms (tSNPs) at the CYP46 gene were genotyped. Results After adjustment for age, gender, education and socioeconomic status, there was no significant association between those with and without APOE4 genotype on Memory performance. On the contrast, CYP46 gene polymorphisms was significantly associated with logical memory performance ($p=0.0006$). Conclusion The CYP46 gene polymorphisms may play an important role in episodic memory performance and SNP4-AA genotype may be a risk factor for memory impairment.

1p36 balanced translocation vs. monosomy 1p36: Different means to a similar end? *R. E. Schnur, C. Rennig, L. B. Coffey, J. Martin, J. Keenan* Dept Pediatrics/Genetics Div, Cooper Univ Hosp, Robt Wood Johnson Med School, Camden, NJ.

We identified three individuals from two families with features of the monosomy 1p36 syndrome who have apparently balanced translocations with 1p36 breakpoints. Patient 1 is a 20 month old girl with developmental delay, neuronal migration and myelination anomalies. She has a 46,XX,t(1;2)(p36.11;q37.3) karyotype and no detected 1p36 deletion with a 1,800,000 clone microarray (Affymetrix,Inc/LabCorp). (She has a 91 kb duplicated 2q37 CNV.) She has microcephaly, full cheeks, small palpebral fissures, nevus of Ota, and 4th finger camptodactyly. Her mother's karyotype is normal. Patients 2 and 3 are an Amerasian mother/son pair [Karyotypes:46, XX,t(1;8)(p36.3;q24.11) and 46,XY,t(1;8)(p36.2;q23)]. The mother has moderate mental retardation, poor eye contact, reduced affect, tremor, but a normal EEG and brain CT. She has microbrachycephaly, short stature, and mildly distinctive facial and skeletal features. She lost a 3 mo old daughter with IUGR, oligohydramnios, and unbalanced chromosomes. Patient 3 has no DNA gains or losses identifiable by two BAC-based CGH panels of 607 (LabCorp) and 4685 clones (Signature Genomics). Additional studies are planned. He had mild IUGR at birth, oligohydramnios, hypotonia, cryptorchidism, early myopia, and global delays. He strongly resembles his mother in personality and facial features, including deepset eyes and prominent nose with broad nasal tip. He has short stature, but a normal head circumference, scoliosis and mild skeletal changes, including 5th finger camptodactyly. In summary, our three subjects all have partial features of the monosomy 1p36 syndrome without demonstrable deletions. Although most previously reported balanced 1p36 translocation carriers are phenotypically normal, a few are not (e.g., Hussain et al. 2000). This report illustrates the difficulties in genetic counseling for individuals with apparently balanced translocations involving 1p36. It also demonstrates the continuing necessity of performing karyotypes *in addition to* CGH for all individuals who exhibit developmental and physical variations.

Identification of a new gene involved in cleft lip using the molecular analysis of an apparently balanced chromosome translocation. *HG. Kim[1], K. Norris[2], AS. Kulharya[2], LC. Layman[1]* 1) Section of Reproductive Endocrinology, Infertility, & Genetics, Dept Ob/Gyn, Institute of Molecular Medicine and Genetics, Medical College of Georgia; 2) Depts. Pediatrics & Pathology, Medical College of Georgia, Augusta, GA.

Cleft lip and/or palate (CL/P) is a congenital developmental anomaly known to have a strong genetic component. CL/P occurs either as an isolated malformation (nonsyndromic) or in association with other developmental anomalies (syndromic). It affects approximately one in every 500 births worldwide, making it the one of the most common major birth defects. Isolated CL/P is genetically heterogeneous and its genetic ethiology has been investigated extensively for many years. Although mutations in genes such as MSX1, TBX10, PVRL1, IRF6, FGFR1, and SUMO1 explain the most commonly encountered etiologies in CL/P, they only constitute about 20% of the molecular basis in these patients, suggesting that other genes have to be involved in the pathogenesis of CL/P. In a number of genetic diseases, structural chromosomal changes that segregate with the disease phenotype have served to map causative genes to specific chromosome regions. In this regard, Mendelian cytogenetics refers to the association between chromosomal rearrangements and single gene disorders. De novo chromosome translocations have been most widely used for the mapping and cloning of disease genes, in which translocation breakpoints provided important information about the gene location. To identify a new CL/P gene by positional cloning, we investigated a male cleft lip patient with an apparently balanced de novo translocation t(10;14)(p14;q31). CGH arrays did not detect additional chromosomal rearrangements, and by performing FISH we narrowed the 10p14 translocation breakpoint to 11.1 Mb containing 39 known genes between RP11-164N21 and CTD-2382H16. The breakpoint of 14q31 was refined to 14.7 Mb , which harbors 66 genes between RP11- 929J14 and RP11-353F5. Thus further refining of both breakpoint regions in our balanced translocation patient is warranted to identify a new causative gene for CL/P.

Co-transcription of genes into single transcripts: Another regulatory mechanism for gene expression in vertebrates. *T. D. Taylor, T. P. Srivastava, V. K. Sharma, N. Kumar, T. Takeda, R. Ozawa, M. Mushiake, R. Okumura, Y. Nishida, T. Fujikake* MetaSystems Research Team, RIKEN Advanced Science Institute, Yokohama, Kanagawa, Japan.

Co-transcription of two distinct genes (child genes) into a single transcript (conjoined gene) has not been well explored. Either it is a somewhat rare phenomenon, or the current methods of genome annotation are not sensitive enough to identify such genes. Towards this we have designed a new computational algorithm "Conjoin" for the identification of conjoined genes in any genome given its messenger RNA or EST information. Applying Conjoin to the human genome, we have identified nearly 750 conjoined genes of variable lengths, some with multiple isoforms. We have so far confirmed the existence of more than 200 of these conjoined genes using RT-PCR and sequencing. Thus, it appears that these conjoined transcripts are arising out of novel functional requirements and are not merely artifacts of transcription. However, the underlying mechanism controlling the formation of such conjoined genes in human and other vertebrate genomes is yet unexplored. In order to confirm the presence of conjoined genes in other vertebrates, we implemented the Conjoin algorithm on both the mouse and chimpanzee genomes. The number of conjoined genes in mouse is far less than that in human, even though there is roughly the same amount of mRNA/EST data available. Thus it appears that the conjoined genes might be performing some novel functions and are contributing to human complexity as compared to other lower organisms. The 5 and 3 flanking regions of the child genes were analyzed to search for the presence of any alternate or common regulatory elements that might be controlling the formation of conjoined genes. Ten regions in the human genome were selected which satisfy the minimum requirement for the formation of a conjoined gene and where no prior evidence existed. In eight of these selected regions existence of a conjoined gene could be confirmed. Finally a comprehensive database of all the human conjoined genes was designed to provide a repository of these specialized genes with detailed information about each gene.

PNA based microarray for microRNA expression profiling. *J. Choi, H. Park* PANAGENE, 816, Tamnip-dong, Yuseong-gu,Dea jeon, Korea.

MicroRNAs (miRNAs) are 22-25 nt non-coding RNAs that play a critical role in many important biological processes, including development, differentiation, apoptosis, metabolism, viral infection, and cancer. Therefore it is important to monitor the expression of miRNAs. PNA (Peptide Nucleic Acids) is synthetic DNA mimics in which the backbone is replaced by repetitive units of N-(2-aminoethyl) glycine. PNA probes have stronger affinity and greater specificity than DNA probes for binding to RNA. These remarkable hybridization properties of PNA suggest that PNA probes may be efficiently incorporated into microarrays. We described a novel PNA based microarray (PANArrayTMmiRNA) that take advantage of highly sensitive and accurate analysis of miRNAs expression profiles. We optimized the protocols about PNA probe design, PNA probe printing, and hybridization conditions of PNA probes-miRNAs. We demonstrated that PNA probes were accurately discriminated human miRNAs with high sequence homology and similar sizes to other miRNAs. Our data showed very low cross hybridization for miRNAs differing by single nucleotide such as human let7 family (a~i), has-mir196 (a and b), and has-mir10 (a and b). Also PANArrayTM miRNA can detect small amount of total RNA with 98% correlation between independent replicates. These results suggest that PANArrayTMmiRNA is a powerful tool to analyze miRNA expression profiles with rapid throughput and high fidelity.

Next Generation Sequencing of the Escherichia coli O55:H7 Genome and Comparison with the Closely Related Enterohemorrhagic E. coli O157:H7. *M. Rhodes[1], P. Vatta[2], C. Cummings[2], L. Wong[2], M. Barker[1], J. Ziegle[1], L. Jones[1], J. Chin[1], P. Brzoska[2], M. Furtado[2], O. Petrauskene[2]* 1) Molecular & Cellular Biology Division, Applied Biosystems, Foster City, CA; 2) Applied Markets R&D, Microbiology group, Applied Biosystems, Foster City, CA, USA.

Detection in the food supply of pathogenic E. coli, particularly strains that cause hemorrhagic colitis (HC), has become a public health priority. The O157:H7 serotype of E. coli has been responsible for most HC outbreaks to date, so detection of this type is critically important. The ideal assay must detect O157:H7, but not any other serotypes, including the vast majority of commensal E. coli that are not pathogenic. E. coli O157:H7 is very closely related to the O55:H7 serotype, which does not frequently cause HC outbreaks. Numerous lines of evidence indicate that O55:H7 is the nearest phylogenetic neighbor of O157:H7, making the design of O157:H7-specific assays challenging. The E. coli O55:H7 genome sequence would be a valuable tool for identification of assay target sequences unique to O157:H7, but no such sequence was available. To this end, the genome of one E. coli O55:H7 strain, and another O157:H7 strain, were sequenced by oligonucleotide ligation and detection using the next-generation AB SOLiD platform. Comparison of the O55:H7 and O157:H7 genomes identified 500 kb of sequence that is present on the O157:H7 chromosome and absent or divergent in O55:H7. Comparison of these putative O157:H7-specific sequences against the publicly available genome sequences of other pathogenic and non-pathogenic E. coli and Shigella strains identified regions that are conserved beyond the O157:H7 lineage, further narrowing the list of putative assay design targets. The short time requirement (two to three weeks from library construction to sequence) and deep coverage obtained (>60X), makes the SOLiD system ideally suited for microbial genome sequencing when a closely related reference genome sequence is available. This method can be sufficiently robust to permit genome sequencing of a reference organisms nearest phylogenetic neighbors.

Sequence and structural variation analysis of a human genome revealed by next-generation sequencing of paired-end libraries. *F. M. De La Vega[1], H. Peckham[2], SS. Ranade[2], S. F. McLaughlin[2], C. C. Lee[2], Y. Fu[2], Z. Zhang[1], F. Hyland[1], R. T. Koehler[1], A. A. Antipova[2], J. M. Manning[2], C. L. Hendrickson[2], L. Zhang[2], E. T. Dimalanta[2], J. K. Ichikawa[2], A. Bashir[3], V. Bansal[3], V. Bafna[3], G. Costa[2], K. McKernan[2]* 1) R&D, Applied Biosystems, Foster City, CA; 2) R&D, Applied Biosystems, Beverly, CA; 3) Dept. of Computer Science, University of California, San Diego, CA.

Ultra-high throughput sequencing allows the analysis of human genomes to discover genetic variation that could have implications in health and disease. We analyzed the genome of an anonymous individual of African origin obtained by with the Applied Biosystems SOLiD System, which allows sequencing single or paired 25-50bp reads of 10^8-10^9 templates on a single array containing beads with clonally amplified templates. We developed fragment and paired-end libraries with insert sizes ranging from 600bp to 3.5kb for a HapMap Yoruba sample (NA18507). We collected over a billion sequencing reads amounting to a total of 32 Gbp of sequence, and obtaining an average 12X haploid sequence coverage and 130X clone coverage. After aligning those reads to the hg18 reference assembly we observe over 95% of the genome bases covered with a read or more and 99.45% of the genome spanned by a paired end clone. A novel error correction technique improved the accuracy of the aligned reads to >99.95%. With such accuracy, our results suggest that over 90% heterozygote identification can be efficiently achieved at 15X coverage levels, by comparison with known HapMap genotypes. We discovered over 2 million SNPs and 117 thousand small indels (1-10bp). The analysis of the distance and orientation of the paired end reads allowed the identification of over 40,000 putative insertions and deletions ranging from 50bp to several Kb. We also predict 50 inversions and 4 gene fusions resulting from deletions, two of which have been previously reported. Depth of coverage analysis allows the inference of copy-number variants. Our results provide guidance for future studies to discover sequence and structural variants in human populations and cancer with short-read next generation sequencing.

Genome-wide linkage and large-scale association studies identify novel variants on chromosome 4q34-35 for type 2 diabetes. *M. Park[1], K. Kim[1], M. Go[1], H. Lee[1], K. Kim[1], K. Kimm[1], Y. Cho[2], H. Lee[2], H. Kim[1], J. Lee[1], K. Park[2]* 1) Division of Structural and Functional Genomics, Center for Genome Science, National Institute of Health, Seoul, Korea; 2) Genome Research Center for Diabetes and Endocrine Disease, Clinical Research Institute, Seoul National University Hospital, Department of Internal Medicine, Seoul National University College of Medicine, Seoul, Korea.

Type 2 diabetes mellitus (T2DM) has strong environmental and genetic etiologic determinants, which are likely to interact in a complex fashion to influence the clinical course of the disease. Identifying the genetic variants that increase risk of T2DM has been a formidable challenge. We conducted genome-wide linkage analyses for T2DM in 388 affected individuals (274 sib-pairs) from 171 Korean families. Of those who had overweight (BMI 23), we found the evidence of linkage on chromosome 4q34-35 and confirmed with multi-point NPL score=2.81 (p = 0.002) through fine-mapping analysis. We also carried out association analyses with SNPs in the region of the linkage signal to identify genetic variants that predispose to T2DM from 2,641 independent individuals. We detected T2DM association signals and confirmed the replicated T2DM susceptibility variants through analyses of large samples of 17,099 individuals from multiple populations. Our systematic search from genome-wide linkage and association studies demonstrate that a replicated linkage peak for T2DM on chromosome 4q34-35 contains at least two novel T2DM-predisposing genes. Our findings suggest that novel T2DM-predisposing genes regulate mechanisms linking overweight to insulin resistance and T2DM, emphasizing independent contribution of multiple variants.

Tissue-specific DNA methylation of the IL2RA promoter: a role for epigenetics in IL2RA expression? *J. Field[1], M. Ehrich[2], M. O'Hely[3], T. Speed[3], J. P. Rubio[1]* 1) Neurogenetics Laboratory, Howard Florey Institute, Carlton, Victoria, Australia; 2) SEQUENOM, 3595 John Hopkins Court, San Diego, CA; 3) Division of Bioinformatics, The Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia.

Interleukin-2 receptor alpha (IL2RA), also known as CD25, is expressed by regulatory T cells and is also up-regulated by CD4+ T cells in response to activation stimuli. The IL2RA gene has recently been identified as a susceptibility gene in both multiple sclerosis (MS) and type 1 diabetes. To determine whether IL2RA expression is epigenetically regulated, we investigated DNA methylation of a 771 bp region of the IL2RA gene, corresponding to the proximal promoter and exon 1, in neural tissue and peripheral blood mononuclear cells (PBMC) from MS patients and controls . We observed a marked tissue-specific difference in relative methylation (Sequenom MassARRAY) of CpG dinucleotides in the proximal promoter region, which contains conserved (PRRI/PRRII) regulatory elements, in neural tissue (high methylation) compared to PBMCs (low methylation). CpG dinucleotides flanking the proximal promoter displayed high levels of relative methylation in both tissues. There was no statistically significant difference in DNA methylation between MS patients and controls in either tissue type. Bisulphite-sequencing confirmed the relative methylation analyses and demonstrated heterogeneity of methylation patterns. In concordance with the relative methylation studies IL2RA expression was reduced in neural tissue compared to PBMC. In conclusion, these data show that tissue-specific DNA methylation of IL2RA is associated with differential gene expression, and this may have consequences for the pathogenesis of autoimmune diseases.

High throughput screening of genes involved in Parkinsons disease using an Affymetrix CustomSeq re-sequencing array. *J. P. Rubio[1], E. J. Wilkins[1], W. Wang[2], T. F. Cowie[3], K. Kotschet[4], M. O'Hely[5], T. P. Speed[5], M. K. Horne[1]* 1) Howard Florey Institute, Melbourne, Victoria, Australia; 2) Stanford Genome Technology Center, Palo Alto, CA, USA; 3) Applied Genetic Diagnostics, National Neuroscience Facility, Department of Pathology, The University of Melbourne, Victoria, Australia; 4) St. Vincent's Hospital, Melbourne, Victoria, Australia; 5) The Walter and Eliza Hall Institute of Medical Research, Melbourne, Victoria, Australia.

Parkinsons disease (PD) is a common neurodegenerative disease, affecting 1% of people over the age of 60. Advances in our understanding of PD have come from the identification of genes (PD genes), and mutations therein, which cause disease. It is now recognized that common (SNP) variation in some PD genes is associated with risk in late-onset sporadic disease. Our aim was to produce both a research and clinical diagnostic tool for PD to enable the investigation of DNA sequence variation in the known PD genes. Conventional sequencing methods were considered too expensive so we turned to Affymetrix CustomSeq technology. In all 185 DNA sequences, comprising 44.3 kb from 16 different genes, including SNCA, Parkin, UCHL1, DJ-1, PINK1, LRRK2, were tiled onto an array. Exons, exon/intron borders, 5 and 3 untranslated regions were also included for most genes. We found that sequence data from the PD GeneChip was both reproducible (from samples analysed in duplicate) and accurate, compared to Sanger sequencing on an ABI 3730 DNA sequencing machine. DNA samples for 100 individuals have been sequenced to date, including PD patients with either an early age-at-onset of disease (55 y.o.), a family history of disease (2 affected relatives) and/or sporadic PD, and healthy controls. A summary of these sequence data will be presented. In conclusion, Affymetrix CustomSeq technology provides a robust and cost-effective approach for high-throughput DNA sequencing, and presents as an attracted alternative for a broad range of DNA sequencing applications including mutation screening and targeted re-sequencing of small (<300 kb) chromosomal regions.

Association of familial autism with imprinted locus on 7q32. *E. Korvatska[1], G. D. Schellenberg[1,2]* 1) Division of Gerontology and Geriatric Medicine, Department of Medicine, University of Washington, Seattle, WA; 2) Veterans Affairs Puget Sound Health Care System.

Background: A strong genetic component of autism is being approached by linkage analysis, candidate gene association studies, and, most recently, by analysis of copy-number variations and whole-genome associations. Given multiple linkage signals found across the human genome, most findings cannot be replicated on different populations. Linkage to 7q is by far the most robust finding in autism. Recently, linkage signal on 7q32 was replicated in two independent studies including ours. Objectives: To search for susceptibility genes within the large genetic interval (20 cM) identified by linkage we performed association analysis of selected candidate genes. Methods: 350 multiplex autistic families were subjected to transmission disequilibrium test. Results: We detected an association with ~220 kb interval situated right under the linkage peak. Signals arose from multiple SNPs comprising several haploblocks with the largest one extending over 100 kb. The associated region contains 5 protein-coding genes, and 4 of which are expressed in the brain. At least 3 of 5 candidate genes are imprinted in human and mouse. Mutations affecting coding sequences/splice sites of 7q32 candidate genes have been searched by sequencing of 100 patients with autism and 100 control individuals. Conclusion: In association study aimed to dissect 7q31-32 susceptibility locus, we have identified a new candidate region containing a cluster of imprinted genes. The associated region is narrowed down to ~200 kb comprising 5 protein-coding gene candidates and 1 miRNA.

Evolution of spinocerebellar ataxia (SCA) genes in primates. *L. Wang[1], X. Hu[1], S. Ji[1], H. F. Liu[1], C. Huan[1], N. Kozuki[2], T. Kurosaki[2], S. Ueda[2]* 1) Medical & Animal Gen, Inst Gen, Chinese Academy Sci, Beijing, China; 2) Department of Biological Sciences, The University of Tokyo, Tokyo, Japan.

Spinocerebellar ataxia (SCA) is an inherited disorder of brain function. More than 25 types of SCA have been described. The basic defect in many types of SCA is an expansion of a CAG triplet repeat. Our previous study on *SCA* 1 gene demonstrated no repetitive structure in the region corresponding to human CAG repeats in prosimians and New World monkeys like in rodents, perfect repeats in Old World monkeys, and interrupted repeats in hominoids. Comparative analysis on secondary structures of primate *SCA* 1 transcripts suggests human prototype was built in the common ancestor of simians (Gene 373:23(2006)). To know systematically how human has acquired the CAG triplet repeats encoding poly-glutamine tracts, we farther investigated *SCA* 2 >, *SCA* 3, *SCA* 6, *SCA* 7, *SCA* 12, and *SCA* 17 genes in various species of primates. For *SCA* 3, CAG repeat expansion might have occurred in the common ancestor of primates. Both synonymous and non-synonymous interruptions have frequently occurred in the common and each lineage of primates. Different from the Q->H interruption of *SCA* 1, various types of non- synonymous (Q->K, Q->P, Q->E, and Q->L) interruptions were found. For *SCA* 17, CAG repeat expansion was widely observed in mammals, and there were a wide variation in the number of repeats. Synonymous interruptions have more frequently occurred in the common and each lineage of primates. Intriguingly, non-synonymous interruption was observed neither in all of the primates nor in all of the mammals examined, except for owl monkey and dog. Synonymous interruption might be more advantageous than non-synonymous interruption for its function as TATA-binding protein. The results show that glutamine expansion in *SCA* 17 is an evolutionarily old variation that happened in the common ancestor of mammals. Glutamine expansion in *SCA* 3 is suggested to occur immediately after divergence of Order Primates, showing an intermediate feature between *SCA* 1 and *SCA* 17. Together with the data for the other *SCA* genes, we show here evolutionary background of glutamine expansion is greatly different from each *SCA* gene.

Analyzing quantitative phenotypes of Alzheimer's disease using Principal Components of Heritability Association Tests (PCHAT). *W. L. A. Fung[1], M. B. McQueen[2], M. S. Albert[3], R. E. Tanzi[4], D. L. Blacker[1]* 1) Epidemiology Dept, Harvard School of Public Health, Boston, MA; 2) Inst for Behavioral Genetics, University of Colorado at Boulder, Boulder, CO; 3) Neurology Dept, Massachusetts General Hospital, Boston, MA; 4) Neurology Dept, Johns Hopkins University, Baltimore, MD.

Background: The extreme multiple testing problem of genome-wide association studies (GWAS) has necessitated the development of statistical methods with high power in the search for additional Alzheimers disease (AD) susceptibility genes. We previously reported on the use of neuropsychological test scores as quantitative phenotypes related to AD that may increase the statistical power for identifying AD genetic risk factors (McQueen et al, Am J Med Genet B, 2007). Power may be further enhanced by reducing multiple phenotypes to a single phenotype with maximal heritability, as is implemented in the newly developed Principal Components of Heritability Association Test (PCHAT) by Klei et al (Genet Epidemiol, 2007). The present study aimed at examining the performance of PCHAT in longitudinal neuropsychological testing data in non-demented subjects. Methods: Using data from 367 community-recruited subjects with and without memory problems, we used PCHAT to analyze the association between performance of 13 neuropsychological tests at baseline and at 1st follow-up visit (adjusted for age, sex, education) with the known AD gene APOE and five other putative AD polymorphisms (SNPs). Results: APOE-4 showed evidence for association with the empirically-derived optimal phenotype including CVLT, digit span backward and Trail Making Test A cross-sectionally (P=0.018) and longitudinally (P=0.022). Interestingly, a promoter in insulin degrading enzyme gave similar results. These P-values (corrected for multiple genotypes and phenotypes) were similar in magnitude to the P-values for the individual neuropsychological tests found in McQueen et al (uncorrected for multiple testing). Conclusions: This is the first application of PCHAT to the study of a complex genetic disorder in unrelated individuals to our knowledge. These preliminary results suggest that this approach has promise in the search for additional AD genes using GWAS.

Genetic and Molecular Evaluation of Idiopathic Male Infertilty: An Indian Perspective. *S. Abid, A. Maitra, D. Modi, J. Gokral* Molecular Endocrinology, NIRRH (ICMR), Mumbai, Maharashtra, India.

Idiopathic male infertility is an enigma, as the cause is still not understood. Genetic and molecular evaluation is being used as tools in identifying the etiology causing male infertility. Mammalian spermatogenesis is a complex cascade of events wherein the testis specific genes and their associated proteins play an important role. Objective of this study was to assess 1. Hormonal profile, frequency of Y chromosome microdeletions and phenotype/genotype association in men with non-obstructive azoospermia (NOA) and severe oligozoospermia (SOA). 2. Gene and protein expression in infertile and fertile testicular tissue. We screened 560 infertile males who attended the institutes male infertility clinic and enrolled 210 men with NOA and SOA. Informed consent was obtained from all subjects and institutional ethics committee approved the study. The frequency of Y chromosome microdeletions observed was 2.8%, which is unusually low and AZFb was the most frequently deleted region, which showed a severe phenotype of azoospermia. Hormonal evaluation revealed that FSH and LH levels were significantly higher in NOA as compared to SOA and serum Inhibin B was significantly low. Severe testicular phenotype such as sertoli cell only syndrome was seen in NOA group as compared to SOA. Men with Yq microdeletions had normal levels of FSH, LH and Inhibin B whereas testosterone levels showed significant decrease. The expression of Y chromosome specific genes RBMY and DAZ was studied using fertile and infertile human testicular tissue and human spermatozoa to understand their role in aberrant spermatogenesis. Low expression of DAZ mRNA was seen in infertile men as compared to fertile. RBMY mRNA and protein expression was also decreased in men with aberrant spermatogenesis. Overall the study shows low prevalence of Yq microdeletions and emphasizes that altered expression of gene and proteins could cause aberrant spermatogenesis leading to idiopathic male infertility. This can thus form an adjunct in evaluating and offering better clinical management to the infertile couple.

Leptin As A Correlate Of Polycystic Ovary Syndrome And Associated Obesity. *M. Pusalkar, P. Meherji, J. Gokral, S. Chinnaraj, A. Maitra* Molecular Endocrinology, NIRRH (ICMR), Mumbai, Maharashtra, India.

Polycystic Ovary Syndrome (PCOS) is one of the commonest endocrinopathies affecting about 5-10% of women in their reproductive age. It is marked by anovulation manifested as amenorrhea, clinical and/ or biochemical evidence of hyperandrogenemia and presence of cystic ovaries on ultrasound. Genetic basis of PCOS is now well established and candidate gene approach is being widely used to study the etiology of PCOS. As obesity is one of the hallmark features of PCOS it is logical to speculate that leptin, the product of obesity gene, could play an important role in pathophysiology of the syndrome. Leptin is mainly synthesized in adipose tissue and is now known as a hormone of reproduction. There is an increasing evidence to show that leptin plays a crucial role in ovarian function via its autocrine- paracrine action. Thus role of Leptin in ovarian dysfunctions such as PCOS has evoked considerable research interest in recent years. The present study was carried out to assess role of leptin in etiology of PCOS and associated obesity. The specific objectives were to compare leptin levels in control and PCOS subjects and to screen leptin gene for putative variations and its association with the phenotype. A total of 100 PCOS subjects and 100 controls were screened. Average BMI as well as plasma levels of leptin were significantly high in PCOS cases as compared to controls ($p<0.0001$). When the two groups were sub-classified as lean and obese, leptin levels were found to be significantly associated with obesity as well as PCOS. Analysis of coding regions in all the study subjects did not show any variations. A novel variation A>G in exon 2 was also identified (GeneBank Accession number DQ054472). In untranslated Exon 1, the polymorphism G>A was observed at -19 position. Polymorphic A allele was found to be associated with increased leptin levels in both control as well as PCOS subjects. The increase was significant in the PCOS group ($p<0.05$) suggesting the confounding effect of certain factors intrinsic to PCOS on this polymorphism.

Heterogeneity of the Genetic Relationship Between C-Reactive Protein and Insulin Resistance Measures. *J. Cui[1], X. Guo[1], L. J. Raffel[1], A. Xiang[2], X. Su[1], Y. Liu[1], L. Zung[1], K. D. Taylor[1], T. A. Buchanan[2], Y.-D. I. Chen[1], J. I. Rotter[1]*
1) Medical Genetics, Cedars-Sinai Med Ctr; 2) USC, Los Angeles, CA.

C-reactive protein (CRP) is an inflammatory biomarker shown to be associated with insulin resistance (IR) and to predict the occurrence of type 2 diabetes and cardiovascular (CVD) events. In an earlier study, we examined the relationship between CRP and diabetes/CVD risk factors, including IR, in a family cohort of Hispanic Americans (HA) at risk for both hypertension and the metabolic syndrome. Significant genetic correlations were found between CRP and several IR measures, including a positive correlation with fasting insulin (FI), and a negative correlation with glucose infusion rate (GINF) during a euglycemic clamp. We assess here the genetic basis of the correlation between CRP and IR measures using the same HA family sample. A 10 cM genome-wide linkage analysis was conducted in 618 individuals from 160 families for CRP, FI, and GINF on non-diabetic offspring who underwent detailed phenotyping. We then performed bivariate linkage analysis for CRP vs. FI, and CRP vs. GINF, to map the common genes underlying both CRP and IR measures,. The maximum LOD score from univariate linkage analysis for CRP was 1.68 on chr.19 (101cM). The greatest evidence for linkage was found for GINF on chr.6 (LOD = 2.82 at 0cM), followed by LOD=2.24 on chr.20 (62cM) for FI. A second linkage peak for GINF was observed on chr. 2 at 267cM (LOD=1.58). The bivariate linkage analysis revealed a maximum LOD score of 2.0 for CRP vs. GINF at the chr.6 locus, with the second highest LOD = 1.61 on Chr.2 (267cM). The maximum LOD for CRP vs. FI was 1.48 at 62cM on chr.20. Even though the LOD scores are modest, these results suggest there are common quantitative trait loci (QTL) underlying CRP and GINF on chr. 6 and 2, while a different potential common QTL for CRP and FI is located on chr. 20. These findings 1) help explain the previously identified different genetic correlations between CRP and GINF compared to CRP and fasting insulin and 2) demonstrate the existence of heterogeneity in the genetic relationships between CRP and various insulin resistance measures.

Genome-wide screening for submicroscopic chromosomal aberrations by High density oligonucleotide array (Affy 6.0) in children with unexplained mental retardation (MR) and dysmorphic features. *A. Tsai[1, 2], L. H. Li[2], F. J. Tsai[3], T. C. Chen[3], C. Wang[3], I. C. Chou[3], C. F. Chang[2], T. P. Chuang[2], J. Y. Wu[2], Y. T. Chen[2]* 1) Div Clinical Gen & Metabolism, Childrens Hosp, Denver, CO; 2) IBMS, Academia Sinica, Taipei; 3) China Medical University.

Genome-wide array is a novel technology that allows for the detection of DNA copy number changes not previously detected by conventional cytogenetic techniques. In this study, high density oligonucleotide chip Affymetrix Genome-Wide Human SNP Array 6.0 was applied to a series of children with previously undiagnosed syndromic developmental delay. Methods: The patients were enrolled from 10 tertiary care hospitals in Taiwan seen by clinical geneticists. All subjects had prior karyotype; some with neuroimaging studies without a diagnosis. All patients were re-evaluated by one clinical geneticist for final entry of array studies. Abnormalities were confirmed by qPCR. Patient and parents were ascertained concurrently with three chips performed and analyzed as a trio set to distinguish between pathogenic and familial non-pathogenic variants. Results: 23 patients were enrolled to date; 12 patients were excluded due to recognizable single gene syndromes or clearly need to rule out neurological or metabolic conditions. On array analysis, 4 of 11 children (36%) have de novo causally related chromosomal aberrations sized from 3.2 MB to 11 MB. One has complex rearrangement involving sub-telomeric region of 1p36.3: deleted from 1p36.32 to pter and duplicated from 1p36.32 to 1p36.31. The rests were noted to have 1q41-42 deletion (5.2 MB) and novel submicroscopic 16q13 deletion and 5q11.2 deletions. Discussion: while most recent data revealed aCGH, among targeted, lower resolution SNP arrays, has an etiologic yield of 13-18%; or 9-14% superior than G-banding; our data is significantly higher than the expectation. Our data revealed the diagnostic value of applying this technology subsequent to detailed history, physical examination, and targeted laboratory testing for other single gene disorders. Our data also demonstrated complex rearrangement which would have been missed by traditional FISH or low resolution SNP/BAC arrays.

Linkage Analysis of a Large Hematological Cancer Family. *D. I. Perera[1], E. M. Tegg[1], J. Stankovich[1,2], R. Thomson[1], J. Silver[2], K. Marsden[3], R. M. Lowenthal[3], C. Flowers[1], R. McWhirter[1], A. Banks[1], A. Piaszczyk[1], J. Panton[3], M. Bahlo[2], S. Foote[1], J. L. Dickinson[1]* 1) Menzies Res Inst, Hobart, Tasmania, Australia; 2) Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia; 3) The Royal Hobart Hospital, Hobart, Tasmania, Australia.

Familial aggregation studies provide strong evidence of a genetic basis for hematological malignancies such as leukemia, lymphoma and myeloma. Furthermore, multiple types of hematological malignancy often occur within individual families. This suggests that relatives may inherit a common predisposition to somatic mutation events in progenitor cells, which results in malignancies in different cell types in different family members. We have performed linkage analysis in a large family from Tasmania, Australia, with 10 cases of hematological cancer. The family includes 7 siblings whose parents are first cousins. Four siblings have been diagnosed with chronic lymphocytic leukaemia (CLL) and 1 with diffuse large B-cell lymphoma. Blood-derived DNA samples from one CLL case, one unaffected sibling, and 7 offspring of the remaining cases were genotyped using Affymetrix 250K SNP arrays, and two regions were identified where patterns of haplotype sharing were consistent with all 5 affected siblings inheriting two copies of a rare susceptibility allele homozygous-by-descent (HBD). Subsequent microsatellite genotyping of DNA extracted from paraffin-embedded tissue has confirmed haplotype sharing in one of the two regions, where four cases have inherited a haplotype HBD and the fifth case carries one copy of this haplotype. A sixth sibling showing early signs of disease has also inherited this haplotype HBD. In addition, two more distantly related cases also appear to carry segments of this risk haplotype. Sequencing and expression studies are underway to identify a causal mutation in this linked region.

Expression profiling of cultured neuronal cells from nasal neuroepithelium reveals involvement of synaptic long-term potentiation and glutamate receptor signaling pathways in schizophrenia. *O. Evgrafov[1], X. Kang[1], B. Wrobel[2], G. Simpson[1], D. Malaspina[3], J. A. Knowles[1]* 1) Psychiatry, Univ Southern California, Los Angeles, CA; 2) Otolaryngology, Univ Southern California, Los Angeles, CA; 3) Psychiatry, New York University, New York, NY.

Expression profiling has been used to identify genes that are differentially expressed between individuals with schizophrenia (cases) and controls. Because gene expression patterns are tissue-specific, cells derived from non-CNS sources are likely to be less relevant to the etiology of the disorder. CNS neuronal cells can be obtained from living subjects, without a brain biopsy, by selective culture of tissue obtained from nasal neuroepithelium biopsies. Using this technique, we have determined the level of expression of ~22,000 core transcripts (mRNAs with annotated full-length CDS regions) in cultured neuronal cells from 5 cases and 5 controls using Affymetrix Human Exon ST 1.0 Arrays, and analyzed the data using software from Partek and Genetrix. The between sample correlation coefficient in controls was very high (r=0.98), substantially greater than that of post-mortem brain samples, and is likely due to the control of environmental conditions that is possible only with cell culture. This reduction in sample variation may allow greater detection of case-control differences due to the decrease in noise. Unsupervised principal component analysis using all core transcripts separates cases from controls. When this data was analyzed using Ingenuity Pathway Analysis software we found significant differences, after correction for multiple comparison using FDR=0.05, in the genes involved in synaptic long term potentiation and glutamate receptor signaling pathway and the processes of neurotransmission and synaptic transmission. These results are consistent with previous studies of candidate genes for schizophrenia. Our study provides further evidence for involvement of these pathways in the etiology of schizophrenia and suggests that the expression profiling of cultured neuronal cells may be a powerful approach in the investigation of molecular mechanisms of neuropsychiatric disorders.

Genome-wide oligonucleotide array-based measurements of copy number variations in samples with abnormal phenotypes. *A. Tsalenko[1], H. Whitby[2], E. Aston[2], C. Hopkins[1], P. Tsang[1], G. Peters[1], D. Bailey[1], L. Bruhn[1], A. R. Brothman[2]* 1) Agilent Technologies, Santa Clara, CA; 2) University of Utah, Salt Lake City, UT.

Copy Number Variants (CNVs) are reshaping our understanding of genome biology and genetics of a growing list of normal and disease-related human phenotypic variations. Previous focused studies of these regions have included different populations of individuals, but all presumably with normal phenotypes. Array CGH analysis of patients with congenital abnormalities has become a main test assay for clinical cytogenetics and genetics laboratories, yet how to interpret normal variants from those of clinical significance has created a dilemma. In this study, we used Agilent Technologies Human Genome CNV microarray set (G4423B, AMADIDs 018897 and 018898) to profile 10 patients selected from a University of Utah database of 1275 patients referred primarily for developmental delay and mental retardation. These patients have been previously profiled by a BAC array platform and are believed to have benign CNVs independent from any clinically significant copy number abnormalities. The Agilent CNV array set of two 244K feature arrays is enriched for coverage of 19,400 previously identified CNVs, in-dels and segmental duplication regions. The median probe spacing varies from approximately 200bp for smaller intervals to as high as 2400bp for larger intervals. Remaining regions of the genome are covered with a median spacing of 27Kb. Using these arrays, we detected 440 to 721 CNVs per patient with a median size of 4.9Kb, compared to 9 calls in a self-self experiment. These CNVs represent 1547 copy number variant regions. Approximately 50% of CNVs called in more than one patient had consistent (up to 1 probe) breakpoints. These findings are in agreement with previous studies of normal samples. In 10 samples, we observed copy number variations in 27 of the 35 most frequent CNVs detected by previous BAC studies. High resolution characterization of CNVs and their boundaries in a wide variety of samples including abnormal patients can help further distinguish benign from clinically significant regions of variation.

Latent class analysis identifies a clinical schizophrenia subtype showing significant linkage to 1q23-25 in Taiwanese families. *E. Holliday[1,2], D. Nyholt[2], B. Mowry[1,2,3]* 1) Queensland Centre for Mental Health Research, Brisbane, Australia; 2) Queensland Institute of Medical Research, Brisbane, Australia; 3) University of Queensland, Brisbane, Australia.

Purpose: This study investigated the utility of empirical clustering techniques to define alternative phenotypes for genetic analyses of schizophrenia. *Methods:* Clinical and genotypic data for 607 Taiwanese affected sibling pair schizophrenia pedigrees was publicly available. For individuals with schizophrenia, latent cluster models were fitted to 13 symptoms commonly associated with a diagnosis of SZ. Models were fitted using SAS statistical software. Posterior probabilities of latent class membership were used to allocate individuals to their most likely latent class. Genomewide linkage analyses were conducted in the subset of siblings concordant for each latent class. *Results:* In the latent class analysis, a four-class model provided the best fit. Sibling concordance of latent class membership was significantly higher than predicted by class probabilities (P=0.004), indicating familial aggregation of group membership. The most prevalent latent class (LC4) described a recognisable subtype of chronically ill schizophrenia patients, with prominent negative symptoms, high disorganisation and poor social/occupational functioning. Due to its prevalence and clinical relevance, genomewide linkage analyses of sibships concordant for LC4 (n=71) was considered the primary analysis. These analyses detected genomewide significant linkage (multipoint LOD = 4.21) to 1q23-25 (genomewide empirical P=0.002). Secondary linkage analyses of sibships concordant for LC1, LC2 and LC3 detected no region with LOD>2. Analyses of the entire dataset yielded a maximum LOD of 1.8 on chromosome 10. *Conclusion:* Empirical clustering techniques may assist with identifying more genetically homogeneous phenotypes for the study of complex disorders. However, large samples are necessary to maintain adequate power in sample subsets. Variants in 1q23-25 may confer risk to a clinical schizophrenia subtype characterised by prominent negative symptoms, disorganisation and poor functioning.

Identification of a novel mutation of OTX2 in a Danish patient with microphthalmia. *K. Grønskov[1], J. Ek[1], A. Sand[1], L. Lavard[2], H. Jensen[1], K. Brøndum-Nielsen[1]* 1) Kennedy Center, Glostrup, Denmark; 2) Glostrup Hospital, Glostrup, Denmark.

The purpose of the study was to investigate OTX2 mutations as the genetic cause of microphthalmia/anophthalmia in a cohort of Danish patients. Microphthalmia is characterized by the presence of a small eye and anophthalmia of absence of an eye within the orbit. The prevalence is reported to be 3 to 30 in 100.000. Microphthalmia/ anophthalmia can occur isolated or as part of a syndrome. The cause can be chromosomal, monogenic or environmental. Of monogenic causes SOX2 is the major gene responsible for 10-15% of the cases. Other genes causing microphthalmia/anophthalmia are OTX2, PAX6, RAX, CHX10 and FOXE3, but only few mutations have been identified for each of these genes. OTX2 is a bicoid-type homeodomain containing transcription factor, expressed during development in the neural and sensory structures, in the eye, ear, nose and brain. The OTX2 gene consists of 3 exons that are alternatively spliced, resulting in at least two protein isoforms of 289 and 297 amino acids. We investigated 23 patients with microphthalmia/anophthalmia for mutations in OTX2. Mutation analysis of OTX2 was performed by direct sequencing of PCR amplified coding regions and MLPA analysis. We identified a two-basepair deletion (c.667_668delGG) in exon 3 of OTX2. This mutation is predicted to cause premature truncation of the protein (p.Gly223TYRfsX28). The patient presented with bilateral microphthalmia, agenesis of the corpus callosum, congenital hypothyroidism, ventricular septal defect, cryptorchidism and asymmetry of the lower extremities.

Detection of human copy number variations using complete hydatidiform moles carrying haploid genomes. *Y. Kukita, K. Higasa, T. Tahira, K. Hayashi* Division of Genome Analysis, Research Center for Genetic Information, Medical Institute of Bioregulation, Kyushu University, Fukuoka, Japan.

Copy number variations (CNVs) of DNA segments in the human genome can confer various phenotypic variations such as risk to complex disease. Because there is an abundance of CNVs with possible population differentiation, cataloging CNV regions for each ethnic group is biomedically important. Available CNV data is far from complete, due to the limitations of detection methods and samples used. We determined a genome-wide high resolution CNV map, using a collection of Japanese complete hydatidiform moles (CHMs) as samples, and analyzing the intensity values obtained by high-density DNA array hybridization (Affymetrix Genome-Wide Human SNP Array 6.0). Using CHMs is advantageous in CNV detection, because the relative change in the hybridization signal caused by CNV is expected to be two-fold larger for CHMs than for usual diploid samples (thus, larger S/N ratio). We also determined autosomal CNVs using the data of diploid samples (HapMap JPT from Affymetrix) for comparison, and found that more CNVs per genome, and shorter mean CNV size in our CHMs than in JPT. Also, many common CNV regions defined by CHMs were not detected as the major common CNV regions by JPT. Comparing our results with those in the Database of Genomic Variants (Toronto), we found that about half of our CNV regions were not reported. In conclusion, the CNV analysis using CHM samples enables sensitive detection of CNVs.

Digital allelotyping revealed tissue specific and allele specific gene expression in human. *K. Zhang[1,5], J. Li[5], Y. Gao[2], B. Xie[2], J. Deng[2], D. Egli[4], E. Leproust[3], K. Eggan[4], G. Church[5]* 1) Department of Bioengineering, UCSD, La Jolla, CA; 2) Virginia Commonwealth University, Richmond, VA; 3) Agilent Technologies, Santa Clara, CA; 4) Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA; 5) Department of Genetics, Harvard Medical School, Boston, MA.

We developed a digital allelotyping method by capturing exonic SNPs from the transcriptome with padlock probes followed with single molecule sequencing. We applied this method to three cell lines (B-lymphocytes, fibroblasts and keratinocytes) established from a human subject in the Personal Genome Project. Approximately 30% of genes showed allele-specific gene expression, among which a fraction of genes are tissue specific, suggesting the presence of tissue specific cis-regulatory polymorphisms. We also applied the digital allelotyping assay on three human embryonic stem cell lines, two of which were derived from the fertilized eggs of a same couple (sibling lines) and hence share 50% genetic identity. The two sibling lines share more genes with the same allelic-specific pattern than with the other genetically unrelated line. Our study highlighted the prevalence of cis-regulatory polymorphisms in the human genome. Many of such polymorphisms could play causative roles in common human diseases.

DATABASE TO SUPPORT THE INTERPRETATION OF HUMAN MISMATCH REPAIR GENE VARIANTS (WWW.MMRUV.INFO). *R. Hofstra, R. Sijmons* Dept Med Gen, UMCG, Univ Groningen, Groningen, Netherlands.

Germline mutations in the mismatch repair (MMR) genes MLH1, MSH2, MSH6 and PMS2 can cause Lynch syndrome which is the most common type of hereditary colorectal cancer. The clinical implications of most missense mutations and small in-frame deletions in these genes are unclear. Clinical classification of MMR gene UVs will be a huge challenge. We have constructed an online database, www.mmruv.info , dedicated to these unclassified variants (UVs) of the MMR genes. It can be easily searched for information on the results of functional assays and other findings, including tumour characteristics (microsatellite instability, immunohistochemical staing of MMR proteins), phenotype (type of cancer, age at diagnosis, Amsterdam criteria, Bethesda criteria), segregation within families and frequency in controls. Results from in silico predictions of pathogenicity will be added as well. Data can be added by the database curators or by users through a web form after a check by the curators. We have formed a collaboration with NICTA (Australia) to automatically mine the literature more thoroughly for relevant MMR gene variant associated publications. Recently formed committees within the International Society for Gastrointestinal Hereditary Tumours (InSiGHT, www.insight-group.org) are working towards collecting data, developing classification algorithms and will act as an expert panel which classifies the MMR gene UVs. Our MMR gene missense database will be one of the tools to support that process. By moving the database and the other two MMR gene LSDBs, that of InSiGHT and of Michael Woods, to the LOVD platform (www.lovd.nl), further steps have been taken to connect and intregrate these databases.

Salla disease without sialuria: extension of the free sialic acid storage phenotype. *F. Mochel[1, 2], B. Yang[2], J. Barritault[3], U. H. Engelke[4], W. S. Benko[5], C. Kaneski[2], F. Seguin[3], R. A. Wevers[4], J. Ding[2], M. T. Vanier[6], F. W. Verheijen[7], R. Schiffmann[2]* 1) Neurogenetics, INSERM U679, Hôpital La Salpêtrière, Paris, France; 2) Institute of Metabolic Disease, Baylor University Medical Center, Dallas, TX, USA; 3) INSERM E0324, Hôpital La Milêtrie, Poitiers, France; 4) Radboud University Nijmegen Medical Center, Laboratory of Pediatrics and Neurology, Nijmegen, The Netherlands; 5) DMNB, NINDS, NIH, Bethesda, MD; 6) INSERM U499, Faculté de Médecine Laënnec, Lyon, France; 7) Department of Clinical Genetics, Erasmus MC, Rotterdam, The Netherlands.

Salla disease is caused by mutations in the SLC17A5 gene, which results in accumulation of free sialic acid in lysosomes. Salla patients present with early onset motor and cognitive deficits. These symptoms are usually well correlated with the brain MRI pattern mostly characterized by diffuse supratentorial hypomyelination, thin corpus callosum and variable degree of cortical and cerebellar atrophy. Elevated urinary excretion of free sialic acid, also called sialuria, is the hallmark of the disease. Using nuclear magnetic resonance spectroscopy (NMRS), we identified two siblings, aged 9 and 16 years, with elevated free sialic acid in cerebrospinal fluid, but remarkably, absence of sialuria. The clinical picture was dominated by mental retardation with minimal motor deficit in the context of mild hypomyelination but no atrophy. Tendon reflexes were decreased while nerve conduction velocity was normal. Despite a mild and unusual phenotype, both patients harbored a homozygous K136E SLC17A5 mutation. Preliminary evidence showed elevated free sialic acid in cultured skin fibroblasts. Sural nerve biopsy contained cytoplasmic endosomal inclusions in Schwann cells, and myelin lipids including gangliosides were analyzed. The presentation of our patients extends the phenotype of free sialic acid storage diseases. SLC17A5 mutations have to be considered in patients with hypomyelination, even in the absence of sialuria. Screening for free sialic acid storage disorders can be further performed by using NMRS in order to determine free sialic acid levels in cerebrospinal fluid.

Mutations of the *SYCP3* gene in women with recurrent pregnancy loss. *H. Kurahashi, H. Bolor, T. Mori, S. Nishiyama, H. Inagaki, H. Kogo, M. Tsutsumi, T. Ohye* Div Molecular Genetics, ICMS, Fujita Health Univ, Toyoake, Aichi, Japan.

Aneuploidy, a chromosomal numerical abnormality in the conceptus or fetus, occurs in at least 5% of all pregnancies and is the leading cause of early pregnancy loss in humans. Accumulating evidence now suggests that the correct segregation of chromosomes is achieved by events occurring in prophase during meiosis I. These include a synapsis between homologous chromosomes, cohesion between sister chromosomes, and meiotic recombination. In our current study, we demonstrate that mutations in *SYCP3*, a gene encoding an essential component of the synaptonemal complex that is central to the interaction of homologous chromosomes, contributes to recurrent pregnancy loss. Two out of 26 women with recurrent pregnancy loss of unknown cause were found to carry independent heterozygous nucleotide alterations in this gene, neither of which was present among a group of 150 control fertile women. Analysis of transcripts from mini-genes harboring each of these two mutations revealed that both affected normal splicing possibly resulting in the production of a C-terminally mutated proteins. The mutant proteins were found to interact with their wild-type counterpart *in vitro* and inhibit the normal fiber formation of the SYCP3 protein when co-expressed in a heterologous system. These data suggest that these mutations are likely to generate an aberrant synaptonemal complex in a dominant-negative manner and contribute to abnormal chromosomal behavior that may lead to recurrent miscarriage. Combined with the fact that similar mutations have been previously identified in two males with azoospermia, our current data suggest that sexual dimorphism in response to meiotic disruption occurs even in humans.

Dual structure of Japanese population and genetic differentiation over the genome. *Y. Yamaguchi-Kabata[1], K. Nakazono[1], A. Takahashi[1], S. Saito[2], N. Hosono[2], M. Kubo[2], Y. Nakamura[3,4], N. Kamatani[1,5]* 1) Laboratory for Statistical Analysis, Center for Genomic Medicine, RIKEN, Tokyo, Japan; 2) Laboratory for Genotyping, Center for Genomic Medicine, Yokohama, Japan; 3) Laboratory for Molecular Medicine, Human Genome Center, Institute of Medical Science, University of Tokyo, Tokyo, Japan; 4) Center for Genomic Medicine, RIKEN, Yokohama, Japan; 5) Institute of Rheumatology, Tokyo Womens Medical University, Tokyo. Japan.

Because population stratification can cause spurious associations in case-control studies, it is important to know whether and to what extent the population is stratified. Furthermore, it is also important to understand how the chromosomal regions have differentiated between subpopulations if the population stratification exists. Our previous study using genome-wide SNP genotypes from 7,003 individuals showed that most of Japanese individuals fell into two clusters, Hondo and Ryukyu (Okinawa) clusters. To clarity how the two subpopulations have genetically differentiated, we examined the level of differentiation and examined the differences in genotype frequencies for genome-wide 270,000 SNPs. The average F_{ST} was 0.003 between Hondo and Ryukyu clusters, while the average F_{ST} between Hondo Japanese and Han-Chinese populations was 0.006. The highly differentiated regions between Hondo and Ryukyu clusters were found in HLA region in chromosome 6, and particular regions in chromosomes 2, 4 and 9. The differentiation in nonsynonymous SNPs was the highest in a SNP in *ABCC11* gene, which is associated with ear wax type. In comparison between Hondo Japanese and Han-Chinese populations, we detected local regions showing high level of differentiation. Among nonsynonymous SNPs examined, the highest F_{ST} was observed at the first methionine codon in *PSCA* gene in chromosome 8. We show the heterogeneity in the level of differentiation over chromosomes among those subpopulations in East Asia, and discuss possibility that high differentiations in particular genomic regions were driven by positive selection.

Gender specific risk alleles and gender interaction modifies the risk for developing asthma at the Vitamin D Receptor (*VDR*) locus. *D. Daley[1], Y. Bosse[2], M. Lemire[3], D. Zamar[1], B. Tripp[1], A. Montpetit[4], A. Becker[5], A. James[6], A. W. Musk[6], L. J. Palmer[7], C. Laprise[8], P. D. Paré[1], T. J. Hudson[3]* 1) University of British Columbia, Vancouver, Canada; 2) Centre de Recherche, Hôpital Laval, Institut Universitaire de Cardiologie et de Pneumologie de lUniversité Laval; 3) Ontario Institute for Cancer Research; 4) Genome Quebec Innovation Centre; 5) University of Manitoba, Canada; 6) Sir Charles Gairdner Hospital; 7) University of Western Australia; 8) University of Quebec at Chicoutimi.

Asthma and atopy are common complex diseases that demonstrate heterogeneity in phenotypic presentation, incidence, and prevalence by age and gender. Asthma is more prevalent in males during childhood and this distribution changes during puberty. It has been postulated that there are gender-specific patterns to the genetic susceptibility of many complex traits. Recent reports of flip-flop effects, with the same associated SNP having opposite allelic associations, have been reported for a number of complex diseases including asthma and the Vitamin D receptor *VDR*. We observed a flip-flip association at this locus between a childhood sample (CAPPS) and an adult persistent asthma sample (SLSJ). We hypothesized that gender may modify genetic effects at the *VDR* locus. We conducted stratified analyses and tested for gender interaction at the VDR locus in 4 study populations: 1) a high risk birth cohort the Canadian Asthma Primary Prevention Study (CAPPS, 549 families), 2) a population-based birth cohort of children from the Study of Asthma Genes and Environment (SAGE, 723 families), 3) a French Canadian founder population (the Saguenay-Lac St. Jean Quebec family based sample (SLSJ, 306 families)) and 4) a population based sample from Australia, the Busselton Health Study population (644 cases and 751 controls). A joint analysis with 5,565 subjects found evidence for gender specific effects and interaction at rs866441(*P*=0.0050) and rs2239179 (*P*=0.0296). There was a protective effect of the GT haplotype in females (OR=0.61) compared with males (OR=1.39), haplotype interaction P-value =0.0025. We postulate that gender effects may be responsible for the flip-flop associations at the *VDR* locus.

Acylcarnitine profiles by tandem mass spectrometry are correlated with physiological condition and nutrition in sever preterm infants. *M. Wada[1], H. Usui[1], M. Yokoyama-Izumi[1], N. Ishige[2], A. Anazawa[2], S. Hosono[1], M. Minato[1], S. Takahashi[1], M. Owada[2], T. Kitagawa[2], H. Mugishima[1]* 1) Pediatrics, Nihon University School of Medicine, Tokyo, Japan; 2) Tokyo Health Service Association, Tokyo, Japan.

Background: Measurement of free carnitine and acylcarnitines allows to detect the several inborn abnormalities of metabolism in neonatal screening. Because available data for sever preterm infants is limited, their acylcarnitine profiles are still unknown and cut off value is discussed. The aim of this study was to characterize the carnitine status in preterm infants with respect to other physiological examinations and nutrition. **Methods:** Seventy-nine preterm infants were divided to three groups by gestational age, 22-27 (A), 28-31 (B), 32-36 (C) weeks and another healthy full-term 30 infants are as control (D). Blood samples were taken at 5th postnatal day and analyzed by tandem mass spectrometry. **Results:** Concentration of free carnitine were significantly (p0.01) lower in group A as very preterm infants compared with group B. While the concentration in group B and C, mature preterm infants, was same level as group D as full-term infants. Free carnitine level showed positive correlation between gestational age (p0.01) and hematocrit (p0.01). Concentrations of total acylcarnitine, short- and long-chain acylcarnitines were significantly (p0.001) lower in group A than in another three groups, but there was no difference of middle-chain acylcarnitines. In addition, in A and B groups, significant (p0.001) positive correlation was found between C3 and amount of protein intake, but not with total protein level in serum. Long-chain acylcarnitines was positively (p0.001) correlated with amount of lipid intake. Furthermore, C5OH in group A was significantly higher than other groups (B: p0.01, C, D: p0.001) and positively (p0.05) correlated with total bilirubin. **Conclusions:** Manifestation of physiological conditions such as anemia, jaundice and information of nutrition is very important to evaluate the carnitine profiles and avoid the false negative or positive of tandem mass screening in very preterm infants.

A two-stage whole genome association study of intracranial aneurysm in a Japanese cohort. *K. Yasuno[1], A. Tajima[1], T. Takahashi[1], A. Hata[2], I. Inoue[1]* 1) Department of Molecular Life Science, Tokai University School of Medicine, Kanagawa, Japan; 2) Department of Public Health, School of Medicine, Chiba University, Chiba, Japan.

Rupture of an intracranial aneurysm (IA) causes subarachnoid hemorrhage, a catastrophic form of stroke. Familial aggregation of IA suggests the existence of genetic risk factors attributing to the formation of IAs. Thus far genetic linkage and candidate-gene association studies were performed in various cohorts, however, consistent loci have not been identified. Most recently, a SNP (rs10757278) on chromosome 9p21 was found to be associated with IA in European populations. We sought to replicate this finding in a Japanese cohort of 773 IA cases and 722 controls, and found a significant association with the same risk allele ($P = 3.8 \times 10^{-5}$, odds ratio = 1.35, 95% confidence interval 1.16-1.57). We also performed a two-stage genome-wide association study in Japanese IA patients and controls (1st stage 300 cases and 200 controls, 2nd stage 460 cases and 460 controls). Completing the 1st stage analysis for over 300,000 SNPs genotyped on the Illumina platform, we performed the 2nd stage genotyping using the Illumina GoldenGate assay for 2,304 tag SNPs which showed *P*-values 0.008 in the 1st stage. Among those SNPs, we have found from a multilocus test of association in the 1st stage samples that two short genomic regions (chromosomes 3 and 17) surpassed a genome-wide significance level of 5×10^{-7}, which supports the inclusion of true positives in the 2nd stage SNPs. The results of the joint analysis of 1st and 2nd stage genotypes are presented.

Replication of *GCKR* polymorphism with triglyceride levels in Hong Kong Chinese population. *C. H. T. Tam, M. C. Y. Ng, W. Y. So, R. C. W. Ma, J. C. N. Chan* Department of Medicine and Therapeutics, The Chinese University of Hong Kong, Shatin, Hong Kong, China.

Background: Recent genome-wide association and replication studies in European populations suggest that glucokinase regulatory protein (*GCKR*) gene located on chromosome 2p23.2-3 are associated with type 2 diabetes and related metabolic traits such as lipids, glucose and insulin levels. *GCKR* regulates glucokinase (*GCK*) which is the first glycolytic enzyme playing an important role in controlling the homeostasis of blood glucose. In this study, we aim to replicate the association of the common *GCKR* rs780094 polymorphism with extensive metabolic traits in Hong Kong Chinese population.

Methods: The single nucleotide polymorphism was genotyped in 594 adults [age mean SD = 41.3 11 years, % males = 45] and 981 adolescents [age mean SD = 15.3 2 years, % males = 47] that participate in a health screening program. Associations of rs780094 at additive model with metabolic traits including body mass index (BMI), waist circumference (WC), percentage body fat as measured by bioelectric impedance (FAT), systolic and diastolic blood pressure, lipids (total cholesterol, triglyceride, HDL, LDL), glucose at OGTT for 0, 60 and 120 min and fasting insulin were assessed by linear regression adjusted for covariates age and sex.

Results: Based on our findings, the reported risk allele T of rs780094 is consistently and significantly associated with an increased level of triglyceride ($P = 9.1 \times 10^{-7}$, geometric mean (95% CI) = 0.90 (0.85 - 0.96) mmol/l for TT carriers, 0.84 (0.81 - 0.87) mmol/l for TC carriers, 0.77 (0.74 - 0.80) mmol/l for CC carriers). However, we did not observe any association between rs780094 and other metabolic traits.

Conclusions: In summary, our study support *GCKR* as a susceptibility locus influencing triglyceride levels in Chinese population.

Mitochondrial DNA Instability and Optic Atrophy Plus Phenotypes due to OPA1 mutations. *P. Amati-Bonneau[1,2], D. Bonneau[1,2], ML. Valentino[3], P. Labauge[4], C. Verny[5], A. Furby[6], G. Lenaers[7], L. Iommarini[3], R. Liguori[8], C. Zanna[3], C. Ayuso[9], J. Arenas[10], R. Garesse[11], B. Wissinger[12], R. Schwarzenbacher[13], P. Reynier[1,2], V. Carelli[3]* 1) Dept Biochem & Molec Biol, CHU Angers, Angers, France; 2) INSERM U694, Angers, France; 3) Dipartimento di Scienze Neurologiche, Università di Bologna, Bologna, Italy; 4) Service de Neurologie, Centre Hospitalier Universitaire de Nîmes, Nîmes, France; 5) Département de Neurologie, Centre Hospitalier Universitaire dAngers, Angers, France; 6) Service de Neurologie, Centre Hospitalier de Saint-Brieuc, Saint-Brieuc, France; 7) INSERM U583, Institut des Neurosciences de Montpellier, Universités de Montpellier I et II, Montpellier, France; 8) Institute of Neurological Sciences, National Research Council - Mangone, Cosenza, Italy; 9) Servicio de Genética. Fundación Jiménez Díaz. CIBERER, ISCIII, Madrid, Spain; 10) Centro de Investigación, and Servicio de Neurología, Hospital Universitario 12 de Octubre, CIBERER, ISCIII, Madrid, Spain; 11) 11Departamento de Bioquimica Instituto de Investigaciones Biomedicas "Alberto Sols" CSIC-UAM, Facultad de Medicina, Universidad Autonoma de Madrid, CIBERER, ISCIII,Madrid, Spain; 12) Molecular Genetics Laboratory, University Eye Hospital Tuebingen, Germany; 13) Structural Biology, University of Salzburg, Austria.

Mutations in OPA1, a dynamin-related GTPase involved in mitochondrial plasticity, have been linked to autosomal dominant optic neuropathy(ADOA, MIM 165500). We here report on eight patients from six ADOA families with sensorineural deafness, ataxia, axonal sensory-motor polyneuropathy, chronic progressive external ophthalmoplegia and mitochondrial myopathy with cytochrome c oxidase negative and ragged red Fibers. We demonstrate that these patients all harboured variable amounts of multiple deletions of mtDNA in their skeletal muscle. All six probands also harboured heterozygous missense mutations in the OPA1 gene. We show that certain OPA1 mutations exert a dominant negative effect responsible for multi-systemic disease, closely related to classical mitochondrial cytopathies, by a mechanism involving mtDNA instability.

The responsible genes for low HDL in Turkish coronary artery disease patients. *M. Alikaşifoglu[1], L. TokgÃ¶zoÄ lu[2], B. Volkan-Salanci[1], E. B. Kaya[2], E. Tülümen[2]* 1) Hacettepe University Faculty of Medicine Department of Medical Genetics, Ankara, Turkey; 2) Department of Cardiology.

Background and aim: The aim of this study is to determine the genetic basis of low HDL in patients with angiographically documented CAD, and HDL <40 mg/dl compared to healthy controls with normal HDL. Methods: A total of 296 patients (M/F: 161/135) were included in the study group. Angiographically, 61% of the patients were diagnosed as CAD and 27% had low HDL (<40mg/dL). The analysis of 16 SNPs from 11 genes including lipid metabolism (Apolipoprotein (Apo) E, CETP, LPL, hepatic lipase, ABCA1, Apo A1) and inflammation (IL6, IL6 rec, IF rec, PPAR and UCP2) were carried out in a total of 200 patients. 7 SNPs were analyzed using TaqMan probes and 9 SNPs with PCR-RFLP. A subset (30/27 case/control) of the total setup was genotyped by 10K Affymetrix GeneScanner. The genotyped samples SNPs were analyzed in terms of allele frequencies, population stratification, and compliance with Hardy-Weinberg equilibrium. The whole genome analysis of the cohort will be completed. Results: HDL levels were higher in B2 allele carriers of CETP Taq1b polymorphism (Kruskall Wallis test (KWt) p: 0,007). Frequency of ApoE2 allele was higher in patients without CAD (X2 p: 0,007) and low LDL levels were observed in E2 allele carriers (JT test p: 0,047). Trigliseride levels were highest in A-allele carriers of ApoA1 mspI polymorphism (KWt, p: 0,042). Both LDL and total cholesterol levels were higher in TT genotype of UCP2 (rs660339) polymorphism (KWt, p: 0,022 and p: 0,032, respectively). ABCA1 rs2230808 GG genotype frequency was higher in control group, compared with CAD patients (X2 p:0,012). Microarray analysis revealed following SNPs to be significantly important: rs1598978 (chr12), rs3887413 (20p12.2, LOC728450), rs1587099 (chr8), rs955639 (chr3), rs1451371 (chr7, DOPA decarboxylase), rs1389455 (chr11, TMEM16C) (for all SNPs p<0.001). Conclusions: The relations of the SNP genotypes and serum lipid levels were correlated with the literature. The SNPs that were found to be significant after microarray analysis and their role on pathophysiology of CAD should be further evaluated.

Association of Vav3 polymorphism with coronary artery disease. *J. Lee[1], E. S. Shin[1], E. Y. Cho[1], Y. Yoo[1], J. lee[1], J. H. Jang[1], J. Lee[2], Y. Jang[3]* 1) DNA Link, Inc, Seoul, Korea; 2) Clinical Nutrigenetics/Nutrigenomics Lab, Department of Food & Nutrition, College of Human Ecology, Yonsei University, Seoul, Korea; 3) Division of Cardiology, Cardiovascular Genome Center, Yonsei Medical Institute, Yonsei University, Seoul, Korea.

Vav proteins contain multiple function motifs and are involved in various cellular signaling processes. Vav3 , a quanine nucleotide exchange factor (GEF) for Rho family GTPases, was an essential factor regulating the homeostasis of the cardiovascular system. Vav3-deficient mice exhibited tachycardia, systemic arterial hypertension and extensive cardiovascular remodeling. We conducted three stage Genome-wide association analysis to identify the association of CAD with VAV3 polymorphism. In stage 1, We screened random SNP(about 100 SNPs) in Vav3 using affymetrix 500K chip in 290 control and 230 CAD Korean population. Next second stage, we analyzed 12 SNPs that linked SNPs with significant SNPs of stage 1 analysis in additional sample set(1087 control vs 1172 CAD) using Molecular inversion probe technology. Then we analyzed cSNP that LD with leading intronic SNP using TaqMan analysis. The risk allele of intronic SNP has a prevalence of approximately 31%, with the risk increased 82.5% in recessive model in combined analysis(stage 1+ stage2, p=8.19 x10-6). The intronic SNP had pairwise r2 values of 0.78 with the cSNP. The risk allele frequency of cSNP is 0.26 in control and 0.30 in CAD. The OR of CAD was 1.69(95% CI, 1.27-2.25) in recessive model. The p values was 3.01 x10-4. The haplotype anlaysis also showed the significant association with CAD(p=2.91 x10-5). But we observed no interaction on the traditional CAD risk factors with cSNP in controls. This result indicates that VAV3 can play a role in the pathogenesis of CAD.

Genome-Wide Association Studies Using RapidMiner. *V. Milanov* Department of Mathematics and Computer Science, Fayetteville State University, 1200 Murchison Road, Fayetteville, NC 28301.

A current challenge of genetic epidemiology is dealing with large numbers of genetic factors. To address this problem, genome-wide association studies (GWAS) explore the human genome in an attempt to identify common genetic factors that influence health and disease. The selection of a reduced set of genetic factors in GWAS can yield insight to biological processes, increase prediction accuracy of diseases, and improve patient care. In this study, we employ the RapidMiner data mining environment to reduce an initial pool of SNP markers in the simulated rheumatoid arthritis data available from Genetic Analysis Workshop 15. Our results indicate that RapidMiner is an effective GWAS tool that can identify disease-associated SNPs with high probability.

Left Ventricular Outflow Tract Obstructions: family data and NOTCH1 mutations. *W. S. Kerstjens-Frederikse[1], R. F. M. Berger[2], Y. J. Vos[1], R. M. W. Hofstra[1]* 1) Dept Genetics, University Medical Center Groningen, Groningen, Netherlands; 2) Dept Pediatric Cardiology, University Medical Center Groningen,Groningen, the Netherlands.

Left ventricular outflow tract obstructions (LVOTO) are often familial. Early detection of (latent) LVOTO or increased familial risk can prevent unexpected cardiac death. To analyse the percentage of familial cases all new and known patients with left sided anomalies seen after april 1st 2006 by the department of pediatric cardiology (170) were offered genetic counselling. Thirty-one patients refused genetic counseling, the others were seen by the same clinical geneticist. In 44 an aortic valve stenosis was diagnosed, in 21 a bicuspid aortic valve without stenosis, in 28 a hypoplastic left heart, in 67 an aortic coarctation and in 10 other left sided anomalies. In 17 patients the family history combined with ultrasound of first degree relatives revealed a LVOTO and in another 19 probands a relative with yet another congenital heart defect was found. The first mutations in NOTCH1 were published in 2005 by Garg et al. in two families with bicuspid aortic valve and other heart defects, and so far two small series showed mutations in approximately 4% of the patients. In our patient group, sequencing has been finished in 40 patients, and 3 mutations have been found. We conclude that LVOTO is often familial and most pedigrees are compatible with autosomal dominant inheritance with incomplete penetrance. NOTCH1 mutations are found in a small percentage of familial and non-familial cases.

Estimation of association P-value at untyped SNP using the publicly available information on pairwise linkage disequilibrium. *J. Ohashi* Dept Human Gen, Grad Sch Med, Univ Tokyo, Tokyo, Japan.

In most of disease-gene association studies, a limited number of single nucleotide polymorphisms (SNPs) in a candidate gene are typed, and many SNPs remain untyped. Here we propose a method for estimating association P-values at such untyped SNPs using the publicly available information on pairwise linkage disequilibrium (LD) between typed and untyped SNPs in the reference population (e.g., HapMap population) in which both SNPs have been typed. A computer simulation revealed that the proposed method showed higher statistical power if untyped SNP to be estimated was directly associated with the disease susceptibility, compared to the case that association tests were performed only for typed SNPs. In addition, the inflation of type I error rate was negligible when the LD between typed and untyped SNPs is strong. Since our approach is mathematically simple, the proposed method would be a practical tool for assessing the necessity of further analysis for untyped SNPs in the candidate gene.

Spinocerebellar ataxia type 6 (SCA6) is associated with small 1A-calcium channel protein aggregates containing expanded polyglutamine in the cytoplasm and the nucleus of human Purkinje cells. *K. Ishikawa, T. Ishiguro, M. Takahashi, T. Amino, H. Mizusawa* Dept Neurology, Tokyo Med & Dental Univ, Tokyo, Japan.

SCA6 is an adult onset neurodegenerative disease caused by a small expansion of trinucleotide (CAG) repeat encoding polyglutamine in the 1A voltage-dependent calcium channel gene (*CACNA1A*). This polyglutamine stretch lies near the carboxyl(C-) terminus of the channel protein. Recently, the C-terminal fragments of the 1A & 1S-calcium channel proteins have been shown to translocate to the nucleus, and have a role in transcriptional regulation in normal condition. To understand the SCA6 pathogenesis, we undertook this study to see the intracellular expression of C-terminus of the 1A-calcium channel protein in human brains. A new rabbit polyclonal anti-1A-calcium channel protein antibody that recognizes polypeptide near the C-terminus was generated. The specificity of this antibody was confirmed by checking recognition of recombinant 1A-calcium channel protein on western blot. Immunohistochemistry was undertaken by the ABC method on formalin-fixed paraffin-embedded tissue sections of control (n=6) and SCA6 (n=4) brains. In controls, immunoreactivity was generally seen in the cytoplasm, but not in the nucleus of any neurons. In SCA6 brains, however, small aggregates were seen specifically in their Purkinje cells. No aggregates were found in any other neurons of SCA6 brains, or in other control brains including other polyglutamine diseases. Aggregates were small (approximately 0.5 to 1.5 m in diameter), but were numerously present in the cytoplasm of SCA6 Purkinje cells. In addition, some aggregates were also found in the SCA6 Purkinje cell nuclei. Theses small aggregates were mostly recognized by anti-expanded polyglutamine antibody *1C2*, but not by an antibody against the C-terminal end of the 1A-calcium channel protein (A6RPT-C; Hum. Mol. Genet. 8: 1185-93, 1999). The present study demonstrates that small aggregates, which consist with the 1A-calcium channel protein containing expanded polyglutamine, are formed both in the cytoplasm and the nucleus of SCA6 Purkinje cells.

Genotype-phenotype correlation of ocular manifestations in cardio-facio-cutaneous (CFC) syndrome. *S. P. Shankar[1], T. L. Young[2], K. A. Rauen[1]* 1) Medical Genetics, University of California San Francisco, San Francisco, CA; 2) Center for Human Genetics, Duke University, Durham, NC 27710.

CFC syndrome is a congenital disorder involving multiple organ systems characterized by cardiac defects, distinctive craniofacial appearance and cutaneous abnormalities. Mutations in genes of the Ras/MAPK pathway *BRAF, MEK1,* and *MEK2* have been identified as causal of the CFC syndrome. In a cohort of 25 mutation positive CFC individuals, 22 had mutations in *BRAF*, 2 had mutations in *MEK1* and 1 patient had a *MEK2* mutation. 21/25 (85%) patients reported ocular involvement; of these strabismus (85%), refractive errors (66%), nystagmus (50%), and optic nerve hypoplasia (23%) were the most commonly reported manifestations. These ocular features were seen in mutations of all three genes. All patients with Q257R, the most common mutation in *BRAF* had strabismus. 5/22 patients with mutations distributed across both the CR1 and protein kinase domains of the *BRAF* gene exhibited optic nerve hypoplasia. The only patient with a *MEK2* mutation, F57C had bilateral cataracts in addition to nystagmus, strabismus, myopia, and optic nerve hypoplasia exhibiting the most severe ocular phenotype. In this cohort of patients, we did not identify any definitive genotype-phenotype correlation; however, the study was limited because of the very small number of patients with *MEK1* and *MEK2* mutations. The high occurrence of ocular abnormalities such as strabismus and optic nerve hypoplasia suggests that the Ras/MAPK pathway plays an important role in human ocular alignment control, and in the development of the optic nerve. Investigations on therapeutics targeting the Ras/MAPK pathway are underway. Further characterization of the ocular manifestations in a larger number of mutation positive CFC patients is important to determine definitive genotype-phenotype correlations and to develop potential treatment strategies.

Cumulative Association of Five genetic variant with coronary artery disease in Korean population. *E. Y. Cho[1], M. Y. Park[1], S. Kim[1], H. Y. Jang[1], M. H. Yun[1], J. lee[2], Y. Jang[3], J. Lee[1]* 1) DNALink Inc, Seoul, Korea; 2) Clinical Nutrigenetics/Nutrigenomics Lab, Department of Food & Nutrition, College of Human Ecology, Yonsei University, Seoul, Korea; 3) Division of Cardiology, Cardiovascular Genome Center, Yonsei Medical Institute, Yonsei University, Seoul, Korea.

Coronary artery disease (CAD) is a major cause of death in both Asian and Western countries. We conducted two-stage genome-wide association study in 230 CAD and 290 controls followed by 3000 single nucleotide polymorphisms (SNPs) were tested for confirmation in 1398 cases and 1378 controls. SNPs in five chromosomal regions- 2p16, 5q31, 9p21, 12p12, 19p13- have been associated with CAD in Korean population. A significant association on chromosome 9p21.3 was independently replicated in the Korean samples. P=1.034 x 10-7 This is the same locus with the strongest association signal in a Caucasian population. Then, we evaluated combined association of the 5 SNPs with CAD. We constructed a genotype score on the basis of the number of unfavorable alleles. The genotype score was associated with CAD in models adjusted for covariates including age, BMI(OR=1.354, 95% CI 1.294-1.561, p= 3.757 x10-8). As compared with subjects with a genotype score of 3 or less (48.5% of the control vs 33.5% of the case), subjects with a score of 6 or more (6.5% of the control vs 14.9% of the case) had a increased risk of CAD (OR=3.36, 95% CI 2.50-4.52, p= 5.09x10-8). In high risk group (under age 55 years or over BMI 25), as compared with subjects with a genotype score of 3 or less subjects with a score of 6 or more have a significantly higher risk OR of CAD (OR=3.73, 95% CI 2.46 - 5.65, p=4.93x10-10); and OR=4.04, 95% CI 2.34-6.98, 5.48x10-7) respectively. This result showed genotype score of five validated SNPs was independent risk factor for incident CAD.

Long term outcome of presymptomatic testing in Huntington disease. *A. Durr[1,2,3], M. Gargiulo[1], S. Lejeune[1], M.-L. Tanguy[4], K. Lahlou-Laforêt[5], A. Faudet[1], D. Cohen[6], J. Feingold[1]* 1) AP-HP, Pitié-Salpêtrière Hospital, Department of Genetics and Cytogenetics, Paris, France; 2) INSERM, UMR_S679 Neurologie & Thérapeutique Expérimentale, Paris, France; 3) UPMC Univ Paris 06, UMR_S679, Paris, France; 4) AP-HP, Pitié-Salpêtrière Hospital, Biostatistic Departement, Paris, France; 5) AP-HP, Georges Pompidou European Hospital, Service de Psychologie Clinique et Psychiatrie de Liaison, Paris, France; 6) AP-HP, Pitié-Salpêtrière Hospital, Department of Child and Adolescent Psychiatry, CNRS FRE 2987, Paris, France.

Our study on long-term outcome of 351 presymptomatic testing for HD had two aims: comparison of the psychological well-being and social adjustment of carriers and non-carriers of the mutation, and identification of predictor for bad outcome. We performed a cross-sectional study, those who had motor signs were excluded from the comparison. A structured interview including 5 self-report scales and the MINI (Mini International Neuropsychiatric Inventory) was proposed. We interviewed 119 testees (53%), 62 non-carriers and 57 carriers after a mean delay of 3.7 years (range: 0.32 to 8.9) after their result. Depression was frequent in asymptomatic carriers (58%). The self reported impact of the test showed that 27% of non-carriers did not cope well with a favourable result, and a significant percentage of non-carriers (24%) were depressed during follow-up. Using different variables such as carrier status, sex, age at time of the result, delay between 1st contact and result, marital status, having children, history of depression, sex of the affected parent, time of being aware of the genetic risk, test motivation. Multivariate analysis showed that only previous episode of depression was predictive of depression after genetic testing in both carriers and non-carriers of the HD mutation ($p<0.0001$). Psychological support and psychiatric care should be given to both carriers and non-carriers after presymptomatic testing for HD. Particularly and regardless of the result, a history of depression before the test and previous familial burden of psychiatric events will influence the outcome after the test.

The role of HIF1-alpha and VEGF genes in gastric cancer. *S. Huang, Y. Tsai, Y. Lee, D. Wu, S. Juo* kaoshiung medical university, kaoshiung, Taiwan.

Background and Purpose Gastric cancer still represents a difficult problem in the field of oncology, in terms of morbidity and mortality. Hypoxia-inducible factor 1alpha (HIF-1alpha), and vascular endothelial growth factor (VEGF) are both important factors in the mechanisms inherent to tumor progression. In the process of tumor development, the continuing proliferation of tumor cell will lead to oxygen deficit. For survival, hypoxia tumor cells reinforce the transcription of VEGF by HIF-1alpha to enhance angiogenesis. The aim of this study sought to investigate whether polymorphisms at the HIF-1alpha and VEGF genes are associated with susceptibility to gastric cancer. Methods We conducted a case-control study. All the cases had pathologically approved gastric cancer. The controls were recruited from the patients who visited the gastroenterological clinic for gastric endoscopy. All the controls had gastric biopsy, and all the biopsy samples showed no evidence of gastric cancer. We selected two SNPs (one for each gene) that had significant association with intestinal metaplasia in our unpublished data. The genotyping was performed using the TaqMan technology. Results A total of 95 cases and 316 controls were included in the present study. Men accounted for 50.16 % of the participants and the average age was 55.412.0 year-old .For HIF1alpha-SNP, compared to the common homozygote CC (n=212) the OR for rare allele carriers CA (n=92) and AA (n=12) were 0.98 (p=0.954) and 0.67 (p= 0.462), respectively. Compared with the CA+CC genotypes, the AA genotype had an OR of 1.49 (p=0.4626). In VEGF-SNP, compared to the common homozygote CC (n=201), the OR for rare allele carriers CT (n=93) and TT (n=22) were 1.25 (p=0.42) and 1.13, (p= 0.799), respectively. Conclusions The present study did not show any significant result for the HIF-1alpha and VEGF genes, which may be due to small sample size. More case DNA samples are currently under genotyping and updated results will be presented in the conference.

To assay the association of SNP and gene expression by combining information among ethnic groups. *C. L. Hsiao[1], I. B. Lian[2], C. J. Chang[3], C. S. J. Fann[1,4]* 1) Institute of Public Health, Yang-Ming University, Taipei, Taiwan; 2) Department of Mathematical, National Changhua University of Education, Changhua, Taiwan; 3) Graduate Institute of Clinical Medical Sciences, Chang-Gung University,Tao-Yuan, Taiwan; 4) Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan.

Different gene expression (GE) pattern among ethnic groups is a popular issue in recent genomic researches, and numerous single nucleotide polymorphisms (SNP) have been identified to contribute significantly to differences in GE. Unfortunately, in study of the relationship between SNP and GE with respect to ethnic groups, the associations were estimated and tested in each group separately which could lose power in detecting significant associations. Furthermore, the genotype frequency differences between ethnic groups have rarely been accounted in such studies. Hence, more refined methods are necessary when studying the association of SNP, GE and ethnic groups simultaneously. To address this issue, a general linear model was used to estimate the relationship between genotype and GE with the entire sample. This model assumes that mechanisms of SNP regulation on GE are similar among different ethnic groups. After the distributions of GE conditional on genotypes were estimated, the amount of GE difference between ethnic groups resulted from genotype frequency difference could be measured by a linear estimator. Then, a general linear hypothesis of the SNP causing GE difference among ethnic groups was tested by an F-statistic. Furthermore, the expected genotype frequencies assuming Hardy-Weinberg Equilibrium were calculated to infer the amount of GE difference among ethnic populations. From our simulation study, this method showed higher efficiency in parameter estimation and was more powerful than testing pair-wise association one by one. In addition, this method could be applied in Genetic Genomic studies with respect to binary coding groups such as case-control study.

The use of multifactor dimensionality reduction (MDR) in study haplotype- haplotype interaction: a novel approach to increase efficiency. *A. R. Hsieh[1], I. B. Lian[2], C. S. J. Fann[1,3], C. J. Chang[4]* 1) Division of Biostatistics,Institute of Public Health, Yang-Ming University, Taipei, Taiwan; 2) Deptment of Mathematical, National Changhua University of Education, Changhua,Taiwan; 3) Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan; 4) Graduate Institute of Clinical Medical Sciences, Chang-Gung University, Tao-Yuan,Taiwan.

Most common human diseases are caused by the complex interaction of multiple genetic and environmental factors. Efficient modeling of this complex interaction requires proper statistical methods to reduce high dimensionality. Haplotype based approaches incorporate information from multiple adjacent SNP markers and may have greater power than single-locus analysis when the SNPs are in strong LD with the risk locus. Unfortunately, the number of distinct haplotypes increases rapidly when the number of SNPs increases. Previously, Multiple Dimension Reduction (MDR) has been used to detect interactions between SNPs or tagSNPs. In this study we used an MDR-based approach to explore the feasibility of haplotype-haplotype interactions to overcome high dimension issues. To demonstrate that haplotype-haplotype interactions provide more information regarding disease association than that for tagSNP-tagSNP interactions, we simulated case-control data using six different two-locus epistasis models by using SNaP sofware. Data were analyzed by MDR with a 10-fold cross-validation. For each model, the ratio of correct classifications to the total number in the testing set was obtained to determine the best combination of attributes. These ratios were ranged from 0.48 to 0.56 for tagSNPs compared to 0.65 to 0.76 for haplotypes. Haplotype-haplotype interactions are thus shown to provide more disease-associated information than tagSNP-tagSNP interactions. Finally, a comparison was made between MDR method and logistic regression models that are commonly used to detect gene-gene interactions in various epistasis models. In the 6 epistasis models with 100 replicates that were simulated, the MDR method showed higher statistical power than that obtained from the logistic regression model.

Fifth gene behind MKS adds more information to the ciliopathy puzzle. *J. Tallila[1], E. Jakkula[1], M. Gentile[2], L. Peltonen[1,3,4,5], R. Salonen[6], M. Kestilä[1]* 1) Department of Molecular Medicine, National Public Health Institute, Helsinki, Finland; 2) Genome Informatics Unit, University of Helsinki, Helsinki, Finland; 3) Department of Medical Genetics, University of Helsinki, Helsinki, Finland; 4) The Broad Institute, MIT Boston, MA, USA; 5) The Wellcome Trust Sanger Institute, Hinxton CB10 1SA, UK; 6) Department of Medical Genetics, Väestöliitto, Helsinki, Finland.

Meckel syndrome (MKS, [MIM 249000]) is a lethal malformation disorder characterized classically by encephalocele, polycystic kidneys and polydactyly. Although the frequency varies greatly among populations, MKS represents the most common form of syndromic neural tube defects (NTDs). Recent findings have shown primary cilia dysfunction in the molecular background of MKS, indicating that cilia are critical for early human development. In Finland, the major mutation in the *MKS1* gene explains about 70% of the cases. To locate the causative gene in the rest of the cases we looked for the homozygous regions shared by the affected fetuses without a known mutation. Based on this strategy we were able to identify the sixth locus and the fifth gene, *CC2D2A* (*MKS6*), behind MKS. The major mutation creates a new splice site leading to defective transcript. In addition, we are currently sequencing non-Finnish MKS families and have identified already six novel mutations in *CC2D2A*. Most of them are small deletions that affect the splicing. The biological function of CC2D2A is uncharacterized, but the corresponding polypeptide is predicted to be involved in ciliary functions and it has a calcium binding domain (C2). Immunofluorescence staining of patients fibroblast cells demonstrated that the cells lack cilia, providing evidence for the critical role of CC2D2A in cilia formation. In order to see the metabolic pathways that are disturbed in MKS we have done genome wide expression arrays using RNA from MKS6 fetuses and healthy controls. These analyses as well as further functional studies on the protein localization and function will bring valuable information about MKS and fetal development.

Linkage tests based on ages of onset. *H. Yoon, H. Song* Dept. of Biostatistics, The Catholic University of Korea, Seoul 137-701, Korea.

The occurrence of many diseases is highly age dependent and thus linkage analysis on age of onset trait appears to be important for many chronic diseases to detect the putative gene. Non-insulin-dependent diabetes mellitus is one of these disorders, for which many studies have shown that a single locus may have a major effect on the occurrence of the disease by influencing age of onset. The Haseman and Elston (HE) [Behav Genet 1972;2:3-19] linkage test, which defines the residuals from survival analysis on ages of onset as a continuous outcome, was shown to be powerful by Zhu et al. [Genet Epidemiol 1997;14:711-716] when a genetic effect is expected primarily due to age of onset. We propose several extensions of the HE test which incorporate information on age of onset or age at examination of sib pairs. These tests are derived from the nonparametric methods without assuming any specific distributional form of age of onset, while the residual-based method by Zhu et al. is distribution-dependent. Apart from sib pair-based linkage tests mentioned above, we propose a generalized logrank trend test based on information of age of onset or age at examination of subjects. A simulation study was performed to evaluate the power of these tests under different sampling plans of age groups, when the distributions of ages of onset are assumed to be known. The identification of a gene influencing age at onset would be important as it would lead us to understand disease development and progression and could provide new targets for therapy.

The Case for a Larger HapMap. *E. Eskin[1], N. Zaitlen[2]* 1) Dept Computer Sci, Univ California, Los Angeles, Los Angeles, CA; 2) Bioinformatics Program, Univ California, San Diego.

The HapMap provides a valuable resource to help uncover genetic variants of important complex phenotypes such as disease risk and outcome. Using the HapMap we can infer the patterns of LD within different human populations. This is a critical step for determining which SNPs to genotype as part of a study, estimating study power, designing a follow-up study to identify the causal variants, ``imputing" untyped SNPs, and estimating recombination rates along the genome. Despite its tremendous importance, the HapMap suffers from the fundamental limitation that at most 60 unrelated individuals are available per population. We present an analytical framework for analyzing the implications of a finite sample HapMap. We present and justify simple approximations for deriving analytical estimates of important statistics such as the correlation coefficient r and the non-centrality parameter lambdasqrt{N} from the HapMap. Finally, we use this framework to show that current HapMap based estimates of r^2 and power have significant errors, and that tag sets highly overestimate their coverage. We show that a reasonable increase in the number of individuals, such as that proposed by the 1000 genomes project, greatly reduces the errors due to finite sample size for a large proportion of SNPs.

Contribution of environmental and genetic modifiers to severity of the typical HFE-related haemochromatosis: a multivariate analysis. *V. Scotet, G. Le Gac, I. Gourlaouen, C. Theze, B. Chanu, A.-Y. Mercier, M.-C. Merour, C. Férec*
Inserm U613, EFS-Bretagne, Universite de Bretagne Occidentale, Brest, F-29200 France.

Background/aim: Expression of the common p.C282Y/p.C282Y HFE-related haemochromatosis genotype depends on a balance between accentuating and reducing factors. Some of these factors have been identified, but they have mainly been analyzed independently. Here, we aimed to determine contribution of different environmental and genetic modifiers to total body iron overload. Method: We studied 365 p.C282Y/p.C282Y patients (195 men and 170 women), and we used the iron removed by phlebotomy as quantitative trait (log transformed). We tested the influence of age, gender, alcohol abuse, body mass index, as well as six common variants: the 16189 T>C variant in mtDNA, the -308G>A variant in the TNF-alpha promoter, the well known duplication of the haptoglobin gene (genotypes Hp1-1, Hp1-2 and Hp2-2), and 3 SNPs located in genes involved in regulation of hepcidin synthesis (BMP2 rs23756, BMP4 rs4901474 and HJV rs16827043). Results: Univariate analyses first highlighted the relation between each potential modifiers and severity of the body iron overload. Factors with a p-value lower than 25% were included in a same multiple linear regression model. Then, stepwise elimination procedures were successively performed until a model presenting only significant predictors was found. Predictors of this last model were the male sex ($p<0.0001$), the greater age at diagnosis ($p<0.0001$), the alcohol abuse ($p=0.0008$) and the Hp2-2 genotype ($p=0.0186$). Conclusion: Our results underline the predominant influence of non-genetic modifiers in expression of the p.C282Y/p.C282Y genotype. Future investigations should address the influence of other genetic modifiers, and further look for gene/gene and gene/environment interactions.

Hereditary optic neuropathies share a common mitochondrial coupling defect. *D. Bonneau[1], A. Chevrollier[1], V. Guillet[1], D. Loiseau[1], N. Gueguen[1], M. Pou de Crescenzo[1], C. Verny[2], M. Ferre[1], H. Dollfus[3], S. Odent[4], D. Milea[5], C. Goizet[6], P. Amati-Bonneau[1], P. Reynier[1]* 1) Dept Gen & Biochem, INSERM U694, CHU Angers, Angers, France; 2) Departement de Neurologie,Centre Hospitalier Universitaire, Angers; 3) Laboratoire de Génétique Médicale EA 3949, Université Louis Pasteur, Strasbourg; 4) Service de Génétique Médicale, CHU Rennes, France; 5) Department of Ophthalmology, Glostrup Hospital,University of Copenhagen, Copenhagen, Denmark; 6) Laboratoire de Génétique Humaine, Université Victor Segalen Bordeaux 2, Bordeaux,France;.

Hereditary optic neuropathies are heterogeneous disease characterized by the degeneration of retinal ganglion cells leading to optic nerve atrophy and impairment of central vision. The two most frequent forms of optic neuropathies are Lebers hereditary optic neuropathy (LHON; MIM #535000) and autosomal dominant optic atrophy (ADOA; MIM#165500). LHON, a subacute bilateral optic atrophy, is associated with mitochondrial DNA point mutations that affect various subunits of mitochondrial respiratory chain complex I. ADOA is a progressive, bilateral optic atrophy. Mutations in the optic atrophy 1 (OPA1) gene are implicated in about 60 to 80%of cases. OPA1 encodes for a ubiquitous dynamin-related protein, anchored to the mitochondrial inner membrane involved in mitochondrial plasticity. In some cases, the clinical phenotype is aggravated by additional neurological symptoms, the so-called plus phenotype, and designated as LHON and ADOA. The third form of hereditary optic atrophy involving an identified gene concerns ADOA associated with cataract (ADOAC; MIM #165300). This rare form of optic atrophy is due to mutations in the OPA3 gene, identified as responsible for the Costeffs syndrome (MIM #258501). Like OPA1, OPA3 is located in the mitochondrial inner membrane, but its function is unknown. We found a common coupling defect of oxidative phosphorylation in fibroblasts of 16 patients affected by ADOA, ADOAC and LHON. Interestingly, the energetic defect was significantly more pronounced in LHON and ADOA patients with a more complex phenotype, the so-called plus phenotype.

The association between genetic polymorphism of ACE and hypertension in adults in central Taiwan. *R.-Y. Wang[2], K.-Y. Tung[1], K.-T. Chen[1], C.-J. Lin[3], W.-C. Shy[3], M.-C. Hung[3], C.-Y. Chen[2], T.-N. Wu[1], F.-Y. Wu[1]* 1) China Medical University, Environmental Health, Taichung, Taiwan; 2) China Medical University, Public Health, Taichung, Taiwan; 3) China Medical University, Nursing, Taichung, Taiwan.

The hypertension related cardiovascular disease account for about half of the top ten leading causes of death in recent year in Taiwan. However, hypertension is an important risk factor for cardiovascular disease and is one of the most important public health problems. Blood pressure is regulated by genetic, environmental factors and their interaction effects. The genetic polymorphism (rs1800764) locating in the upstream of angiotensin-converting enzyme (ACE) gene was suggested to have an impact on hypertension. Therefore, the aim of this study was to evaluate the association between rs1800764 genetic polymorphism of ACE and hypertension in adults aged 40 years in Sinyi village, a remote area of Nantou county in the middle of Taiwan. This is a case-control study. The subjects consisted of 153 hypertension patients and 497 normotensive subjects. Blood specimens were collected to determine the biochemical data, and demographic information were collected by conducting face-to-face interviews with structured questionnaires. The genotypes of rs1800764 were carried out by TaqMan assay. Our results showed that TT, CT, and CC genotypes of rs1800764 were all associated with blood pressure (p0.0001), and also associated with hypertension (p=0.0067). With adjusting age and gender, the analytical results of logistic regression indicated that compared to subjects with TT genotype, the subjects with CT and those with CC genotype, the odds ratios of hypertension were 1.74 (95% CI: 1.12 - 2.71) and 2.48 (95% CI: 1.45 - 4.13), respectively. In this study, we demonstrated that rs1800764 genetic polymorphism on ACE gene would associate with hypertension. Moreover, studies on the effects of rs1800764 or other variants on ACE gene and their interactions with environmental factors on hypertension or cardiovascular disease will be warranted.

CEREBELLAR ATAXIA WITH HYPOGONADISM: CLINICAL, NEURORADIOLOGICAL AND GENETIC FEATURES IN NINE FAMILIES. *C. M. Lourenco[1], C. F. R. Sobreira[2], J. M. Pina-Neto[1], W. Marques Jr.[2]* 1) Medical Genetics, University of Sao Paulo , Ribeirao Preto, Sao Paulo, Brazil; 2) Neurology,University of Sao Paulo , Ribeirao Preto, Sao Paulo, Brazil.

INTRODUCTION The association between cerebellar ataxia and hypogonadism was described in four sibs by Holmes 100 years ago, and has since become known as Holmes type ataxia. At the time of his description, it was not possible to determine whether hypogonadism was hypogonadotropic or hypergonadotropic hypogonadism. Here, we present the clinical data and molecular/biochemical studies of nine Brazilian families with cerebellar ataxia and hypogonadism. MATERIAL AND METHODS All the patients were evaluated in the neurogenetics clinics by geneticists, neurologists and endocrinologists. Brain MRI, ophtalmological exam, EMG/NCV, hormone and biochemical tests, karyotype, muscle biopsy, molecular tests for Friedreich ataxia and for SCAs (types 1, 2, 3, 6 and 7) were performed in the course of the investigation. RESULTS All patients had cerebellar ataxia, but the age of the onset was variable; it was worthy to note that ten patients had early onset ataxia. Consanguinity of parents was noted in two families; five patients had hypogononadism hypergonadotropic. Mental retardation was seen in two unrelated girls with hypogononadism hypergonadotropic. None of the patients had chromosomal anomalies and biochemical screening for CDG disorders was also norma. Molecular tests for Friedreich and SCAs 1, 2 ,3 , 6 and 7 were all negative. Optic atrophy and retinochoroidal degeneration were found in five patients; axonal neuropathy was present in four patients. Cerebellar atrophy with pons or prominent vermis involvement was a constant feature. CONCLUSIONS One family have features consistent with a rare neurological disorder , Boucher-Neuhauser syndrome; the other patients had features that may fit in the Gordon-Holmes phenotype although we believe this entity should not be an homogenous disorder. Thus, the association between cerebellar ataxia and hypogonadism comprise hetereogenous entities whose clinical investigation can elighten the pathological basis of these fascinating neuroendocrinological syndromes.

Haplotype inference of copy number variations. *M. Kato[1], Y. Nakamura[1,2], T. Tsunoda[1]* 1) Center for Genomic Medicine, RIKEN, Yokohama, Kanagawa, Japan; 2) Human Genome Center, University of Tokyo, Minato-ku, Tokyo, Japan.

Extensive studies have recently examined large-scale genetic variants called copy number variations (CNVs) in the human genome, and the universality of CNVs in normal individuals, along with their functional importance, has been increasingly recognized. However, the absence of a method to accurately infer alleles or haplotypes of CNVs from high-throughput experimental data hampers the finer analyses of CNV properties and applications to disease association studies.

We developed two algorithmic frameworks to infer haplotypes/alleles of CNVs. The first algorithm processes unrelated individuals phenotypic copy numbers, which are the total numbers of DNA copies over two homologous chromosomes and are obtained using high-throughput experimental platforms. From these data, the algorithm infers population frequencies of alleles represented as copy numbers in one homologous chromosome. It can also handle phenotypic copy numbers that are ambiguously determined, such as 2 or 3 copies, due to experimental noise. Furthermore, the algorithm can infer population frequencies of haplotypes composed of both copy number alleles and SNP alleles. The second algorithm processes the total numbers of polymorphic bases over two homologous chromosomes within a CNV region for unrelated individuals, such as two counts of polymorphic base A and one count of polymorphic base G, of which the total count is not two but three due to copy number variation. From these data, the algorithm infers population frequencies of complex haplotypes that can yield information on both sequences and numbers of DNA copies, such as three units of DNA copies: ATG, ATG, GTG.

We demonstrated good accuracies of our algorithms using a variety of simulation datasets, and also applied these algorithms to real datasets on the CYP2D6 and MRGPRX1 genes in two HapMap populations. Our algorithms will infer haplotypes of CNVs to support the rigorous analyses of population genetics for CNVs as well as association studies that detect copy-number and nucleotide differences related to phenotypic traits such as disease susceptibilities.

Finnish prostate cancer families have an increased risk to other cancers. *S. Pakkanen[1], M. P. Matikainen[2], E. Pukkala[3], P. Koivisto[1], T. L. J. Tammela[2], J. Schleutker[1]* 1) Laboratory of Cancer Genetics, Institution of Medical Technology, University of Tampere and Tampere University Hospital, Finland; 2) Department of Urology, Tampere University Hospital and Medical School, University of Tampere, Finland; 3) Finnish Cancer Registry, Helsinki, Finland.

Clinical features of families with prostate cancer (PCa) and other malignancies associated with this disease are not well known. A family with PCa is characterized as two or more PCa cases among first degree relatives. The aim of this study was to assess weather primary tumours other than prostate carcinoma aggregate in Finnish PCa families. Based on the national population based Finnish Cancer Registry (FCR), we calculated standardized incidence ratios (SIR) for 5546 members of 202 Finnish families with PCa with confirmed genealogy, either using the first diagnosed PCa among brothers as a single index or multiple indexes. Multivariate cluster analysis was used to separate families with clinically aggressive disease to a separate analysis. The total number of cancers (all sites) among males was 552 (SIR 1.79) in single index group and 234 (SIR 0.94) in multiple index group and among females 205 (SIR 0.98). Females in the sample had more stomach cancer than expected (SIR 1.88, 95% confidence interval 1.19-2.94) when compared general population. Both females and males in the age group 60-80 had more cancer in gallbladder (SIR 2.35, 1.17-4.60). Males in the families with clinically aggressive disease had more gallbladder cancer and small intestine cancer. In a subgroup analysis by age groups there was more breast, colon, kidney and ovary cancer. In most of the families with excess numbers of prostate cancer the disease appears to be site specific. However a suggestive tendency towards gastric and gallbladder cancers was detected. Inherited risk of cancer may be age dependent as in subgroup analysis other cancers have an increased risk. In addition when analyzing families with clinically aggressive disease other cancers are more common. These results suggest that there is an excess of other cancers in families with Pca and the type of cancer depend on the clinical aggressiviness of the PCa.

Hereditary cancer predisposition in a subset of uveal melanoma patients and their family members. *M. Abdel-Rahman[1,2], R. Pilarski[3], S. Ezzat[4], J. LaJeunesse[1], F. Davidorf[1]* 1) Ophthalmology, The Ohio State University, Columbus, OH; 2) Pathology, Menoufiya University, Egypt; 3) Department of Internal Medicine and Comprehensive Cancer Center, the Ohio State University; 4) Department of Public Health, Menoufiya University, Egypt.

Uveal melanoma (UM) is the most common primary intraocular malignancy in adults. The extent of contribution of familial/hereditary predisposition to the development of uveal melanoma is largely unknown. Thus we sought to ascertain the frequency of cancers in patients with UM and their family members to identify the prevalence of hereditary/familial predisposition to cancer in these patients. An unselected series of 112 patients with UM seen in a university-based tertiary referral program were consented to the study. Cancer histories (site and age of diagnosis) were obtained for all first- and second-degree relatives. Observed cancer frequencies were compared to the expected risk for development of the same cancers in the general population, as obtained from the Surveillance Epidemiology and End Results (SEER) database. Out of the 112 probands included in the study 11 (9.8%) had very early onset of uveal melanoma (40 years old at diagnosis), 23 (20.5%) had a history of at least one other primary cancer (excluding non melanoma skin and lung cancers) and 14 (12.5%) had a strong family history of cancer. Only one patient had a family history of a first degree relative with uveal melanoma. For probands the relative risk of cancer were significant for skin melanomas (RR: 8.8, 95% CI: 2.4-22.8), but not for breast cancer (RR: 2.39, 95% CI: 0.77-5.58), colon cancer(RR: 3.26, 95% CI: 0.67-9.52), or prostate cancer (RR: 1.92, 95% CI: 0.62-4.48). In conclusion, although familial uveal melanoma (i.e. multiple individuals in the same family affected with uveal melanoma) is rare, a significant subset of uveal melanoma patients and their family members may have a hereditary/familial predisposition for development of other cancers. Identifying the cancer predisposition gene(s) in these individual is important for proper management of both the patient and their family members.

Different genomic alteration pattern in acral melanoma tumors identified by an MLPA approach. *C. Badenas*[1, 3], *J. A. Puig-Butille*[1, 3], *Z. Ogbah*[2], *C. Carrera*[2], *P. Aguilera*[2], *J. Malvehy*[2, 3], *S. Puig*[2, 3] 1) Biochemistry & Molecular Genetics, Hosp Clinic & IDIBAPS, Barcelona, Spain; 2) Melanoma Unit, Dermatology Department, Hospital Clinic & IDIBAPS, Barcelona, Spain; 3) Centro Investigación Biomédica en Enfermedades Raras (CIBERER), ISCIII. Barcelona. Spain.

Recent studies have revealed distinct genetic patterns between different histopathological melanoma subtypes, suggesting that initiation/progression of melanoma could be attributable to different somatically mutated genes and different chromosomal rearrangements. Gain of CCND1 gene has been detected as a genetic marker of acral lentiginous melanoma (ALM). NRAS and BRAF genes have been described constitutively activated in a high proportion of melanoma tumors. Another locus implicated in melanogenesis process is CDKN2A (9p21) since deletions affecting the 9p21 region have been described in the majority of melanoma samples. The aim of the present study was to characterize the genetic aberrations detected in different subtypes of melanoma by using MLPA technique (9p21 region and set of oncogenes). We included 69 melanoma tumors (8 lentigo malignan -LM-, 4 nodulars -NM-, 40 superficial spreading -SSM- and 17 ALM). BRAF and NRAS mutational status was also studied. No mutations were detected in NRAS. BRAF mutations were detected in 30% of samples, but the frequency differ among the different subtypes, being more frequent in non acral (range 25-37.5%) than acral melanomas (6%). No difference in deletions affecting the 9p21 region was detected. MLPA detected amplifications in at least one region in all LM, NM and ALM, while it was detected in 42% of SSM. Six regions showed a different pattern of amplification between acral and non-acral melanomas. Two of them were statistically significant: 5p25 and 11q13 gains were detected in 31.2%of acral melanomas while only in 6.9% of non-acral (p=0.04, OR:6.1 for both regions). Moreover, MLPA analysis could differentiate three sub-types of acral melanomas: those with amplifications affecting 1p13.2 and 5p25, those with gains in 11q13 and a third subtype in which the 20q13 region was amplified. These results suggest different genomic pathways leading to acral melanoma progression.

Inferring Human Population Structure: STR or SNP? *S. Xu*[1], *L. Jin*[1,2] 1) Chinese Academy of Sciences and Max Planck Society (CAS-MPG) Partner Institute for Computational Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, China;; 2) Ministry of Education (MOE) Key Laboratory of Contemporary Anthropology and Center for Evolutionary Biology, School of Life Sciences and Institutes of Biomedical Sciences, Fudan University, Shanghai 200433, China.

Both microsatellites (STRs) and single nucleotide polymorphisms (SNPs) have played important roles in inferring population structure. With the availability of genome-wide STR and SNP data for the same collection of world-wide human population samples (HGDP-CEPH panel), we now have the opportunity to compare the usefulness of the two types of data in inferring population structure. We selected the same set of 940 unrelated HGDP individuals in which both 783 STRs and 650,000 SNPs were genotyped, and performed both classical (phylogenetic and principal component) analysis and STRUCTURE analysis. We found for all analyses, with the same allele number, SNP data perform better and generate more reasonable results than STR data. Notably, a) SNP data offer superior clustering of individuals and populations; b) the phylogenetic tree reconstructed using SNP data is consistent better with the geographical distribution of populations; c) SNP data reveals fine structures and reasonable admixture pattern in STRUCTURE analysis for both full samples and subset of samples, but STR can not.

Associations among metabolic genes, repair genes and DNA damage of pregnant women exposed to environmental tobacco smoke. *F.-Y. Wu[1], R.-Y. Wang[2], C.-J. Lin[3], C.-Y. Chen[2], H.-C. Tsui[1], C.-C. Yang[1], C.-J. Chan[1]*
1) China Medical University, Environmental Health, Taichung, Taiwan; 2) China Medical University, Public Health, Taichung, Taiwan; 3) China Medical University, Nursing, Taichung, Taiwan.

The aims of this study were: 1) to investigate whether the exposure to environmental tobacco smoke (ETS) in pregnant women would increase their DNA damages, and 2) to examine the associations among DNA damage, polymorphisms in metabolic genes (GST M1, NAT2) and polymorphisms in repair genes (hMLH1) in the pregnant women exposed to ETS. This is a cross-sectional study. The subject consisted of 165 pregnant women (78 passive smokers and 87 non-smokers) recruited from the National Smoking Prevention Project during 2003-2004. Results showed that the mean DNA damage score of the passive smoking group was 88.044.33, which was significantly higher (p0.0001) than that of the non-smoking group of 63.032.3. Carriers of GSTM1 null genotypes, NAT2 slow acetylators and those with the hMLH1 A allele variant were more likely to have a higher degree of DNA damage than carriers of the non-null genotype, rapid acetylators and those with the G allele variant, regardless of whether they were non-smokers (18.4%, 11.1% and 43.8% increase, respectively) or passive smokers (11.5%, 15.6% and 30.4% increase, respectively). Analysis by simple linear regression found that in passive smokers carrying one or less, two or three variant genes resulted in DNA damage scores of 79.9, 91.5 and 111.6 respectively. These were higher than the respective scores of 58.6, 65.7 and 86.8 in non-smokers. Moreover, the level of DNA damage increased as the number of variants in each of the three genes increased. Compared to the group of non-smokers carrying one or less gene variant, there were a significantly greater number of variants in EST group. In conclusion, the associations indeed existed between polymorphisms in metabolic genes and in the repair gene and smoking exposure. Genotypic variants of these polymorphisms did elevate DNA damage for pregnant women exposed to smoking hazard.

A population genetics study of the Familial Mediterranean Fever gene: evidence of balancing selection supports the heterozygote advantage hypothesis. *R. Cagliani[1], M. Fumagalli[1,2], U. Pozzoli[1], S. Riva[1], G. P. Comi[3], G. Menozzi[1], N. Bresolin[1,3], M. Sironi[1]* 1) Scientific Institute IRCCS E.Medea, Bosisio Parini, LC, Italy; 2) Bioengineering Department, Politecnico di Milano, 20133 Milan, Italy; 3) Dino Ferrari Centre, Department of Neurological Sciences, University of Milan, IRCCS Ospedale Maggiore Policlinico, Mangiagalli and Regina Elena Foundation, 20100 Milan, Italy.

Familial Mediterranean Fever (FMF) is a recessively inherited systemic autoinflammatory disease caused by mutations in the MEFV gene. The frequency of different disease alleles is extremely high in multiple populations from the Mediterranean region, suggesting heterozygote advantage. Here, we characterize the sequence variation and haplotype structure of the MEFV 3' gene region (from exon 5 to the 3' UTR) in 7 human populations. In non-African populations, we observed high levels of nucleotide variation, an excess of intermediate-frequency alleles, reduced population differentiation and a genealogy with two common haplotypes separated by deep branches. These features are suggestive of balancing selection having acted on this region to maintain one or more selected alleles. In line with this finding an excess of heterozygotes was observed among Europeans and Asians, suggesting an overdominance regime. In African populations, a minor highly divergent haplotype clade was observed; the deep TMRCA of the whole MEFV genealogy together with an analysis of the gene tree topology indicated that these chromosomes might result from admixture among subdivided archaic hominid populations. Our data, together with the previous demonstration that the MEFV exon 10 has been subjected to episodic positive selection over primate evolution, provide evidence for an adaptive role of nucleotide variation in this gene region. These data therefore support, although indirectly, the heterozygote advantage hypothesis for FMF mutations and suggest that further studies aimed at clarifying the role of MEFV variants might benefit from the integration of molecular evolutionary and functional analyzes.

Bayesian Estimation of Multi-Risk Components in Autism Spectrum Disorders (ASDs). *T. Nishiyama[1,2], K. Takahashi[3], H. Taniai[4], S. Sumi[5], H. Kishino[6], T. Toshiro[3]* 1) Department of Information and Biological Sciences, Graduate School of Natural Sciences, Nagoya City University, Nagoya, Japan; 2) Doctor of Public Health Program in Biostatistics, National Institute of Public Health, Wako, Japan; 3) Department of Technology Assessment and Biostatistics, National Institute of Public Health, Wako, Japan; 4) Nagoya Child Welfare Center, Nagoya, Japan; 5) Nagoya Western Rehabilitation Center for Children with Disabilities, Nagoya, Japan; 6) Laboratory of Biometry and Bioinformatics, Graduate School of Agriculture and Life Sciences, University of Tokyo, Tokyo, Japan.

Background Recently, cytogenetic findings point to a higher incidence of spontaneous mutation including copy number variations (CNVs) in children with sporadic autism. Zhao et al. (2007) found a simple genetic model in which most families fall into two types: a small minority for whom the risk of ASDs is near 50%, and the vast majority for whom their offspring have a low risk, mostly based on samples with at least two ASD children. However, such ascertainment scheme could bias the composition of ASD families. The purpose of this study is to examine how many risk components are found in population-based ASD sample. **Methods** Subjects in the present study ware a cohort of siblings born between 1995 and 1999 who were thoroughly ascertained through at least one proband having an ASD in the catchment area (Sumi 2006). To estimate the risk of producing offspring with an ASD, we choose to model the distribution of the risk as having two to ten discrete components and used a Markov chain Monte Carlo method. **Results** The best-fitted model in DIC(Deviance Information Criteria) was five-component risk model, whose posterior mean of each risk was 0.039, 0.090, 0.253, 0.300, 0.408 in turn, with 0.3 of penetrance in females. **Conclusions** We can interpret the risk distribution of transmitting an ASD trait as continuous rather than discrete, though the risk component of about 0.5 have also been found. Our finding is in disagreement with the previous work (Zhao et al., 2007). This may be due to their use of the biased samples which were ascertained via at least two affected children with an ASD.

Siblings with pure subtelomeric trisomy 9q due to maternal cryptic translocation. *S. Mizuno[1], D. Fukushi[2], R. Kimura[2], T. Kumagai[1], N. Wakamatsu[2]* 1) Dept Pediatrics, Central Hosp, Aichi Human Service Ctr, Kasugai, Japan; 2) Dept of Genetics, Institute for Developmental Research, Aichi Humana Service Center, Kasugai, Japan.

In recent years, subtelomeric rearrangements have been identified as a major cause of multiple congenital anomalies (MCA)/mental retardation (MR) syndromes, and some of newly diagnosed MCA/MR syndromes with subtelomeric rearrangement have distinctive features which can be phenotypically diagnosed. Here we report on female siblings with mental retardation, both showing a distinct phenotype, associated with the same 9q subtelomeric rearrangement due to maternal cryptic translocation. The proposita were borne to healthy unrelated parents with no hisotry of spontaneus abortion. Their birth weight were, 2364 g(-1.4SD), 2874 g(-0.2SD), head circumference were 33cm(+0.2SD),33.5cm(+1.0SD),respectively. Both girls showed a similar phenotype with postnatal growth retardation, moderate developmental delay, relative macrocephaly, slender fingers, and distinctive face with hypertelorism, broad flat nasal bridge, a small nose and round face. The older sibling had congenital heart disease. Routine chromosomal analysis using G- banding alone showed 46,XX in both siblings and inv(2)(p11.2q11.1) in the older. Genome-wide subtelomere FISH analysis revealed additional 9q subtelomeric signal on 13p satelite region. Parental chromosome analysis using G-banding and Genome-wide subtelomere FISH revealed that their mother has the same inversion in chromosome 2, and her 9q subtelomeric signal is translocated on 13p satelite region. By using additional FISH with bacterial artificial chromosome (BAC) probes, we comfirmed the size of trisomic segment to be less than 6Mb. Previously reported cases with 9q distal trisomy were concurred with other diatal monosomy, and deletions are generally more phenotypically penetrant than duplications. To our knowledge this is the first case report with pure 9q subtelomeric trisomy due to maternal cryptic translocation, and the characteristic features of the siblings suggest that subtelomeric trisomy 9q is a clinicallly recognizable syndrome.

A global expression atlas of genes and microRNAs in the developing mouse embryo. *G. Diez-Roux, EURExpress Consortium* Telethon Institute of Genetics & Medicine, Naples, Italy.

The EURExpress consortium has generated a global expression atlas of the developing mouse on sagittal sections from E14.5 wild type embryos by means of ISH with non-radioactive probes and used this data to establish a web-linked, interactive digital transcriptome atlas (www.eurexpress.org). This atlas includes over 15000 expression patterns, which have been thoroughly annotated using over 1400 anatomical terms. A genome-wide analysis revealed that 42% of genes show a regional expression pattern. To determine sensitivity, the EUREXpress data was compared to more high-throughput gene expression methods such as microarray. This analysis revealed that 30% of regional genes are not detected by microarray analysis. This percentage increases to 40% when analyzing genes that show exclusive expression in a specific tissue. The analysis of the EURExpress data also allowed us to identify coexpressed gene clusters shown to be both statistically and biologically significant. The coexpressed clusters seem to have much higher specificity than clusters based upon publicly available microarray data. These data highlight the importance and use of RNA in situ hybridization as an alternative to microarray analysis in large-scale gene expression studies. To complete the global expression atlas we analyzed the entire set of mouse microRNAs (miRNAs) using the same technological platform. As a result we generated the first high-resolution expression study of this class of non-coding RNAs in a mammalian organism. This analysis revealed that of 425 miRNAs analyzed, 45% are non-detectable in the E14.5 mouse embryo while the remaining 55% shows significant expression with both widespread and restricted patterns. We are presently comparing this data to the expression patterns of host and predicted target genes. This analysis will allow improving the identification of bona fide transcriptional targets of these important regulators of gene transcription and translation. In conclusion, EURExpress is a unique resource for scientists worldwide providing researchers with an atlas of gene expression at a cellular resolution, for both coding and non-coding transcripts.

Phenotypic characteristics including neurohistopathologic findings in a child with neuronopathic Gaucher disease type 3B. *H. J. Kim[1], T. H. Lee[2], D. E. Jung[3], S. Y. Jeong[1]* 1) Dept. Medical Genetics, Ajou Univ. Sch. Med., Suwon, Korea; 2) Dept. Pathology, Ajou Univ. Sch. Med., Suwon, Korea; 3) Dept. Pediatrics, Ajou Univ. Sch. Med., Suwon, Korea.

Gaucher disease (GD) is an autosomal recessive, lysosomal disorder caused by mutations in the gene for the -glucocerebrosidase (GBA) enzyme. Progressive neurologic symptoms can develop in some forms of GD, classified as type 2 (acute infantile form) and type 3 (subacute juvenile form). Here we report an autopsy case of a Korean female child with neuronopathic GD type 3B who died of aspiration pneumonia at age 31 months. The patient was presented with anemia, thrombocytopenia, and splenomegaly at age 10 months and diagnosis of GD was made with the demonstration of Gaucher cells (GC) in bone marrow biopsy and the deficient enzymatic activity of GBA. With enzyme replacement therapy of high dose of Cerezyme (>60/kg q 2wks), her clinical pictures of anemia, thrombocytopenia, splenomegaly and general condition improved makeldey in 6months, however, her neurologic symptoms of ocular apraxia and myoclonic seizures become evident. She developed a bouts of preumonia with dyspnea and in spite of antiepileptics, her myoclonic epileptic seizures became intractable requiring mechanical ventilation. Postmortem examination revealed a systemic involvement of brain, lung, visceral organs and bone marrow. The brain showed a diffused global involvement with mutifocal infiltration of GC observed in large number of subcortiacal gray-white junctional perivascular space in cerebrum. In cerebellum, multifocal patchy involvement of GC was observed in the subcortical perivascular space and cortical neuron layers, which is associated with marked neuronal loss, atrophy and gliosis. Furthermore, the brain stem abnormality was absent and hippocampal sclerosis showing marked neuronal loss, astrogial and microglial proliperation without infiltration of GC was observed. The neurohistopathologic findings in this case may contribute to a better understanding of the pathological basis of neuronopathic GD which may lead to better treatment strategy for the neuronopathic GD.

Widespread balancing selection and pathogen-driven selection at blood group antigen genes. *M. Sironi[1], M. Fumagalli[1,2], R. Cagliani[1], U. Pozzoli[1], S. Riva[1], G. Menozzi[1], N. Bresolin[1,3], G. P. Comi[3]* 1) Scientific Institute IRCCS E. Medea, Bosisio Parini, LC, Italy; 2) Bioengineering Department, Politecnico di Milano, P.zza L. da Vinci, 32, 20133 Milan, Italy; 3) Dino Ferrari Centre, Department of Neurological Sciences, University of Milan, IRCCS Ospedale Maggiore Policlinico, Mangiagalli and Regina Elena Foundation, Via F. Sforza 35, 20100 Milan, Italy.

Historically, allelic variations in blood group antigen (BGA) genes have been regarded as possible susceptibility factors for infectious diseases. Since host-pathogen interactions are major determinants in evolution, BGAs can be thought of as selection targets. In order to verify the this hypothesis, we obtained an estimate of pathogen richness for geographic locations corresponding to 52 populations distributed worldwide (HGDP-CEPH panel); out of 26 BGA loci, after correction for multiple tests and for variables different from selective forces, significant correlations with pathogen richness were obtained for multiple variants at 12 loci. Given these findings, we made use of publicly available data (SeattleSNPs Variation Discovery Resource), as well as of extensive resequencing experiments in human global populations, to gain further insight into the evolutionary history of BGA genes. By applying population genetics tests we were able to demonstrate that 5 BGA genes, namely CD55, CD151, SLC14A1, GYPC and BSG, have been subjected to balancing selection, a process, rare outside MHC genes, which maintains variability at a locus. Moreover, we identified a gene region immediately upstream the transcription start site of FUT2 which has undergone non-neutral evolution independently from the coding region. These data indicate that BGAs have been playing a central role in the host-pathogen arms race during human evolutionary history and no other gene category shows similar levels of widespread selection, with the only exception of loci involved in antigen recognition.

3q26 altered region detected in a Spanish wild-type CDKN2A melanoma-prone family. *J. Puig-Butille[1,3], M. Stark[4], C. Badenas[1,3], Z. Ogbah[2], P. Aguilera[2], C. Carrera[2], J. Malvehy[2,3], N. Hayward[4], S. Puig[2,3]* 1) Biocehm. & Molecular Genetics, Hospital Clinic and IDIBAPS. Barcelona, Spain; 2) Melanoma Unit, Dermatology Department, Hospital Clinic and IDIBAPS. Barcelona, Spain; 3) Centro de Investigacion Biomedica en red en enfermedades raras (CIBERER). ISCIII, Barcelona, Spain; 4) Oncogenomics, Queensland Institute of Medical Research (QIMR), Brisbane, Australia.

Familial Melanoma accounts for 10% of melanoma cases. CDKN2A (9p21) and CDK4 (12q14) are two major susceptibility and mutations in these loci are detected in 16% of Spanish MM families being more frequent in families with multiple primary (MPM) cases. However, a high proportion of families do not harbor mutations in these genes. Other regions (1p22 and 1p36) have been related to MM susceptibility. A dramatic Spanish family with different cancers including MM was studied. The index patient developed 7 MM and 3 Basal cell carcinomas. His son developed 3 MM and his sister had breast cancer. Mutational screening of CDKN2A and CDK4 genes was performed. Furthermore, the 9p21 region was interrogated using MLPA in order to identify deletions/amplifications affecting CDKN2A and other adjacent genes. Whole genome analysis from the two MPM cases was carried out by SNP-array chip (Illumina Sentrix HumanHap300 genotyping Beadchip array). No alterations affecting CDKN2A, CDK4 or 9p21 region were identified. In contrast, loss of heterozygosity (LOH) OF 190KB in 3q26.1 region was detected in the index patient. This LOH was not detected in his son, but a larger region (1.6 Mb) in this location presented uniparental disomy (UPD). The region of overlap between father and son comprised 30 kb. Bioinformatics softwares were used to identify known or putative genes in this region. A putative gene (NT_005612.1195) which would codify a gene product similar to CAB99359 has been predicted. CAB99359 is a novel protein with unknown function although it contains EGF-like and laminin G domains. The results presented here suggest a possible implication of the 3q26.1 region in melanoma predisposition. Acknowledgments: Exchange Programme for young researchers funding by genoMEL Consortium.

Genome-wide analysis of microsatellite mutation in humans. *A. Coghlan, W. Whitener, R. Durbin* Wellcome Trust Sanger Institute, Cambridge, United Kingdom.

To investigate the mutation patterns of human microsatellites, we have generated substantial new data on triplet repeat variation in the human population. We have developed a novel bioinformatic method to analyse data from the Trace Archive. We identify repeat-length polymorphisms by finding cases where the length of a repeat locus differs between the reference genome and a matching sequence read in the Trace Archive. Using this approach, we have found evidence of length-polymorphism in 18,503 of the 77,095 human triplet repeat loci. In bacteria and yeast, the rate of expansions and contractions is higher at microsatellites that can form non-B DNA structures such as hairpins and triplexes. For a repeat locus to form such structures, its sequence must fulfil certain requirements. For example, (AAG)n loci consist of (purinepyrimidine)n mirror repeats and this enables them to fold into triplex DNA. We find that the most variable human triplet repeat loci are those predicted to form unusual DNA conformations. For example, (AAG)n loci are significantly more variable than (AAC)n loci, which do not form hairpins or triplexes. This supports the hypothesis that unusual DNA conformations are mutagenic intermediates of microsatellite instability. Some microsatellites are 'fragile sites' susceptible to DNA lesions, and faulty repair of lesions can result in expansions or contractions. In yeast, fragile sites are located in regions that are replicated late during S-phase and are susceptible to DNA breaks. Thus, there may be a link between replication timing and microsatellite instability. Using replication timing data for 1% of the human genome from ENCODE, we found that triplet repeat loci in late-replicating regions are significantly more variable than loci in mid- or early- replicating regions. This suggests that microsatellites replicated later during S-phase have higher mutation rates. We have also developed a method to identify microsatellite length-polymorphism using paired-end Solexa/Illumina sequencing data. With tight insert-size distributions and deep coverage we can identify the majority of microsatellite length-polymorphisms in a sample. This promises to provide a deeper picture of microsatellite length-variation in the future.

Seizures and epilepsy in neurofibromatosis type 1 (NF1): a genotype-phenotype study. *A. L. Gabriele[1], M. Ruggieri[2,6], P. Iannetti[3], A. Patitucci[1], A. Magariello[1], T. Sprovieri[1], R. Mazzei[1], F. L. Conforti[1], C. Ungaro[1,4], L. Citrigno[1,4], M. Muglia[1], M. Clementi[5], A. Polizzi[6], I. Torrente[7], M. Elia[8], L. Pavone[6], A. Quattrone[1,9]* 1) ISN - CNR, Cosenza, Italy; 2) Institute of Neurological Science (ISN), National Research Council (CNR), Catania, Italy; 3) 2nd Chair of Paediatrics, Division of Child Neurology, Institute of Paediatrics, University La Sapienza, Rome, Italy; 4) Department of Neurosciences, Psychiatric and Anesthesiological Sciences, University of Messina, Messina, Italy; 5) Institute of Epidemiology and Medical Genetics, Department of Paediatrics, University of Padua, Padua, Italy; 6) Department of Paediatrics, University of Catania, Catania, Italy; 7) CSS-Mendel Institute, Roma, Italy; 8) Institute for Mental Retardation and Developmental Disabilities, Department of Neurology, Oasi Maria SS., Troina (EN), Italy; 9) Institute of Neurology, University of Magna Graecia, Catanzaro, Italy.

The aim of this study is to define the prevalence of seizures and epilepsy in NF1 and to establish a genotype-phenotype correlation. We investigated twenty-nine patients with NF1 and seizures (including febrile seizures) and/or epilepsy. The calculated population-based: (1) prevalence of febrile seizures (FS) in NF1 was 1.35%, in line with the frequency of FS in the general population (1-3%); (2) prevalence of epilepsy in NF1 (2.9%) was lower than the frequency of seizures in the general population (3%);(3) prevalence of infantile spasms (IS) in children with NF1 (0.76%) was higher than the reported frequency of IS in the general population (0.02- 0.05%); (4) frequency of NF1 in the IS series in two out of the three clinical centres (0.62-0.90%) was lower than the estimated frequencies in the literature (1.5-3.0%). Germ-line mutations were identified in all subjects: three of these alterations were novel. This is the first genotype-phenotype study on seizures and/or epilepsy in the setting of NF1. According to the present findings epilepsy is not associated with NF1. The combination of IS and NF1 is certainly an unusual event in NF1. DNA analysis revealed NF1 gene mutations without genotype-phenotype correlation.

FAF1 is Associated with Cleft Palate and Pierre Robin Sequence. *M. Ghassibe[1], L. Desmyter[1], B. Bayet[2], N. Revencu[1], R. Vanwijck[2], V. Vikkula[1]* 1) Lab Human Molecular Genetics, de Duve Institute, Brussels, Belgium; 2) Centre Labiopalatin, Division of Plastic Surgery, Cliniques universitaires St Luc, Brussels, Belgium.

Nonsyndromic isolated clefts occur in a wide geographic distribution with an average birth prevalence of 1/700. Genetic factors involved in cleft lip with or without the palate (CL/P) are thought to be different from those having a role in cleft palate only (CPO). We have recently reported FAF1 as a new gene responsible for CPO and Pierre Robin sequence (PRS). Moreover, we showed that Faf1 is needed for craniofacial development in human, mouse and zebrafish. In order to replicate our positive association study, we conducted transmission disequilibrium test in an independent series of 160 European families with cleft lip and/or palate. The same FAF1 variant as in our first study was genotyped. In the replication, FAF1 showed positive tendency for association only in the small CPO/PRS subgroup (p=0.09). Pooling together our 500 patients reinforced the earlier association, giving a more stringent p-value of 0.001 for the CPO/PRS subgroup, and non-significant p-value for the CL/P subgroup. In order to identify other genes that contribute to the occurrence of this multifactorial condition, we are testing in parallel association of IRF6 and SATB2, two cleft genes, to the cleft condition in our 500-patient cohort. Preliminary results suggest that, contrary to FAF1, IRF6 predisposes to CL/P, but not to CPO. This illustrates the benefit of testing greater number of patients in complex diseases, and replicating positive associations, in order to well delineate the true predisposed subgroups. Moreover, it confirms that FAF1 and IRF6 play a role in the occurrence of isolated complex clefts, but most likely in distinct pathways. (miikka.vikkula@uclouvain.be).

Construction of research resource repository and molecular genetic study for mental retardation in Japan. *Y. Goto[1], E. Nakagawa[1], K. Takano[1], K. Inoue[1], J. Inazawa[2], H. Okazawa[2], M. Kato[3], T. Kubota[4], K. Kurosawa[5], S. Saitoh[6], E. Nanba[7], N. Matsumoto[8], T. Toda[9], T. Wada[10], Japan Mental Retardation Research Consortium* 1) Mental Retard/Birth Def Res, National Inst Neuroscience, Kodaira, Tokyo, Japan; 2) Medical Research Institute, Tokyo Medical and Dental University, Tokyo, Japan; 3) Yamagata University School of Medicine, Yamagata, Japan; 4) University of Yamanashi, Faculty of Medicine, Yamanashi, Japan; 5) Kanagawa Childrens Medical Center, Kanagawa, Japan; 6) Hokkaido University Hospital, Sapporo, Japan; 7) Tottori University School of Medicine, Tottori, Japan; 8) Yokohama City University School of Medicine, Kanagawa, Japan; 9) Osaka University School of Medicine, Osaka, Japan; 10) 10Shinshu University School of Medicine, Nagano, Japan.

Recent advances in molecular genetics and genome medicine have pushed on with systematic analysis of mental retardation (MR) study, especially on X-linked MR in Europe and USA. Japan is behind because we do not have the system for the research resource repository of many specimen and accurate clinical information available for genetic analysis. Japan Mental Retardation Research Consortium (JMRRC) has organized a research system at NCNP for genetic analysis of mental retardation. In the past 6 years, we have already established the research resource facility and collected samples under informed consent by providing the diagnostic service for known genetic defects and/or chromosomal abnormalities, such as genes for fragile X, MECP2, ARX, ATRX, and subtelomere deletions. As of the end of March in 2008, 208 pedigrees consisted of 103 familial and 105 sporadic cases have been registered. The examined cases (about 100 cases) with positive results include 2 with FMR1 expansion, 1 with FTSJ1 gene mutation, 1 with ZNF41, 1 with PAK3, 1 with OPHN1, 1 with ARHGEF6, 2 with ATRX, 1 with repeat expansion in ARX gene, 5 with duplication of specific regions in X chromosome and 1 with deletion of subtelomeric region. We also performed genetic counseling for the families with the positive result according to the guidelines.

Cleft Palate Caused by a 12q24.33 Amplification. *L. Desmyter[1], A. Ghalamkarpour[1], M. Ghassibe[1], Ch. Labrèze[2], F. Morice-Picard[3], M. Vikkula[1]* 1) Lab Human Molecular Genetics, de Duve Institute, Brussels, Belgium; 2) Unité Dermatologie Pédiatrique, Hôpital Pellegrin Enfants, Bordeaux, France; 3) Unité de Génétique Médicale, CHU de Bordeaux, Bordeaux, France.

Orofacial clefts are the most frequent craniofacial malformations in humans. Occurrence estimates range between 1/500 and 1/2500 births for cleft lip with or without the palate (CL/P) and around 1/2000 births for cleft palate only (CPO). The majority of clefts are isolated, nonsyndromic. The remaining syndromic cases are subdivided into categories on the basis of chromosomal abnormalities, Mendelian single gene syndromes, teratogenic effects and of unknown cause. We performed regular and molecular karyotyping using Affymetrix GeneChip on a total of 200 individuals. In one female patient, we identified a reciprocal translocation affecting the chromosomes (12;22). The translocation was not balanced, since copy number analysis showed a 12q24.33 amplification and a 22q13.33 deletion. The girl was the only affected member of the family and presented congenital progressive lymphedema, mental retardation and facial dysmorphism, indicative of a 22q13 deletion syndrome, also known as Phelan-McDermid syndrome. Interestingly, she had CPO, a feature not linked to this well characterized syndrome. We hypothesize that this is due to the translocation breakpoint on the 12q24.33 amplification site. This locus has not been incriminated in CL/P nor CPO before. FISH analyses are ongoing to characterize these breakpoints. This shows that molecular cytogenetics is a valuable tool for the identification of new genes related to complex diseases. (miikka.vikkula@uclouvain.be).

IDENTIFICATION OF A NOVEL DELETION IN A COUPLE WITH RECURRENT SPONTANEOUS ABORTIONS. *S. Dudeja[1], M. Kumar[1], M. Tanwar[1], S. Venkatesh[1], R. Kumar[1], N. Malhotra[2], R. Dada[1]* 1) Lab for Molecular Reproduction & Genetics, Deptt. of Aantomy; 2) Deptt. of Obstetrics & Gynaecology, All India Institute of Medical Sciences, New Delhi, India.

Recurrent spontaneous abortion (RSA) is defined as three consecutive abortions. The etiology of recurrent abortions is often unclear and may be multifactorial. Majority of the cases remains unexplained. Couples having multiple miscarriages are at risk for carrying chromosomal abnormalities. This report shows the importance of cytogenetic studies in couples with RSA. A couple was referred for cytogenetic analysis with the history of recurrent miscarriages. Pregnancy history, age, occupation, disease information and all other medical records were reviewed. The wife had history of four abortions. The husband had occupational exposure to lead fumes. Various studies have shown that most of the chromosomal abnormalities associated with recurrent abortions are numerical and structural which may adversely affect embryonic development. The chromosomal rearrangements in couples with recurrent abortions were found to be 3.5% in Indian population. There are reports of recurrent abortions with pericentric inversion, translocations, insertions and deletions of various chromosomes. Peripheral blood was collected and lymphocyte cultures were set up for chromosomal analysis. The metaphases were analysed using GTG banding. The wife was cytogenetically normal. A novel deletions in chromosome 1 and 3 has been found in husband, karyotyped 46,XYdel1(q41)(p12q21) 3(q)(26.3-29). This finding illustrates the importance of performing cytogenetics studies on couples having recurrent abortions to prevent couples undergoing physical and emotional trauma. The detection of couples with chromosomal abnormalities can definitely aid in preventing birth of children with congenital malformations. Thus cytogenetic analysis should be done in couples with repeated pregnancy losses or having bad obstetric history.

High-density SNP association and CNV analysis of two Autism Susceptibility Loci. *A. T. Pagnamenta[1], E. Maestrini[2], J. A. Lamb[3], N. Sykes[1], I. Sousa[1], C. Toma[2], E. Bacchelli[2], A. P. Morris[1], A. J. Bailey[4], A. P. Monaco[1], IMGSAC* 1) WTCHG, University of Oxford, UK; 2) Dipartimento di Biologia, Università di Bologna, Italy; 3) Centre for Integrated Genomic Medical Research, Manchester, UK; 4) Department of Psychiatry, University of Oxford, UK.

Autism is characterised by impairments in communication and reciprocal social interaction, restricted interests and repetitive behaviour. The high genetic component inferred from twin studies has led to numerous genetic linkage studies being conducted. The International Molecular Genetic Study of Autism Consortium, has identified regions on chromosomes 7 (AUTS1) and 2 (AUTS5). Although these regions have been further implicated in independent linkage studies, screening of several candidate genes has thus far failed to identify the underlying genetic variants. In this study, 4 Illumina GoldenGate 1536 arrays were designed to optimally capture common genetic variation in AUTS1 and AUTS5. These SNPs were genotyped in 126 families, preselected for identity-by-descent sharing between affected sibs in the AUTS1 and AUTS5 linkage regions, and 188 gender-matched controls. Association analysis implicated several new genes: in the respective family-based and case-control analyses, *PLXNA4* and *IMMP2L* were the most significant genes in AUTS1 whilst *ZNF533* and *NOSTRIN* were identified in AUTS5. Due to the small number of families, association is being assessed in larger replication cohorts. Using the QuantiSNP algorithm, CNVs were detected in *IMMP2L* and *EMID2* in autistic patients and verified on a genomewide SNP platform. Despite inconclusive segregation analysis and presence of similar events in the Database of Genomic Variants, these findings should be extended to larger cohorts with enough power to determine whether these events have subtle effects on autism susceptibility. In conclusion, a high-density SNP association study of these 2 autism susceptibility loci has identified several genes showing genetic association or CNVs in this autism cohort, many of which warrant further investigation. Of particular interest is *IMMP2L*, an inner mitochondrial membrane protein, which was detected in both genetic association and CNV analyses.

Genetic variants on *NRG1* confer susceptibility to Hirschsprung's disease. *C. S. Tang[1,2], M. M. Garcia-Barceló[2], S. S. Cherny[1], P. C. Sham[1], P. K. H. Tam[2]* 1) Department of Psychiatry, Li Ka Shing Faculty of Medicine, The University of Hong Kong, HKSAR; 2) Department of Surgery, Li Ka Shing Faculty of Medicine, The University of Hong Kong, HKSAR.

Hirschsprung's disease (HSCR, aganglionic megacolon), is a congenital disorder characterized by the absence of enteric ganglia in variable portions of the distal intestine. Besides the major HSCR gene, *RET*, and other implicating genes (e.g. *EDNRB*), existing evidence suggests that additional loci contributing to sporadic HSCR exist. To identify these loci, we conducted a genome-wide association study on 200 Chinese HSCR patients and 408 ethnically matched controls. Apart from SNPs in *RET*, the strongest overall associations were found for two SNPs (rs16879552 and rs7835688) located in intron 1 of the neuregulin1 gene (*NRG1*) on 8p12. Replication was carried out on an independent set of 190 HSCR case and 510 control subjects, yielding the combined odds ratios of 1.68 (p=1.80x10^{-8}) and 1.98 (p=1.12x10^{-9}) for the heterozygous risk genotypes of rs16879552 and rs7835688 respectively under the additive model. Significant interaction was also found between *RET* and *NRG1* (p=0.0095), which further increased the odds ratio 2.3-fold to 19.53 for the *RET* rs2435357 risk genotype (TT) in the presence of the *NRG1* rs7835688 heterozygote. The significant association suggests an important role of *NRG1* as regulator of the development of the enteric ganglia precursors.

Phenotype diversity in Iranian cohort of LQTS patients. *K. Banihashemi[1], S. Saber[2], M. Houshmand[3], M. Eftekharzadeh[4], M. Sadre Ameli[5], F. Fazelifar[5], E. V. Zaklyazminskaya[6]* 1) Dep. of Medical sciences, GPEF, Ministry of Science, Research and , tehran, tehran, Iran; 2) Member of genetic society of Iran; 3) National Research Center for Genetic Engineering and Biotechnology (NRCGEB); 4) Tehran Arrhythmia Center; 5) Rajaii Heart Hospital, Tehran; 6) Center for Molecular Genetics, Odintsovo, Russia.

Background: Long QT syndrome (LQTS) is an inherited group of diverse diseases with greatly variable Clinical manifestations characterized by QTc prolongation, syncope and high risk of SCD due to polymorphic ventricular tachycardia. Objective: To characterize the clinical diversity of LQTS patients in Iran and specify features associated with increased SCD risk in this ethnic group. Materials and methods: We analyzed clinical data from 28 Iranian LQTS families (30 affected and 93 clinically unaffected family members) in a follow-up period of 3220 months. The prevalence and intensity of cardiac events like syncope, ventricular tachycardia, cardiac arrest, and SCD estimated and efficiency of beta-blockers therapy as well. Results: Half of patients have received beta-blocker therapy. The prevalence of cardiac events among these patients was 60% (P<0.001). The risk of cardiac events was higher among LQTS with T wave shape abnormality and patients with QTc duration of <500 ms (95% [CI]; P=0.001). BY hazard function analysis other risk factor of SCD was a QT interval corrected for duration of therapy of < 25 months length (95% CI; P=0.01). Conclusion: In Iranian cohort of LQTS patients treated with beta-blockers, there is a high rate (60%) of cardiac events, especially among those had T-wave morphological and quantitative abnormalities and QTc > 500 ms. Beta-blockers had been reported as highly effective in cardiac events risk reduction (to 10-32% in different LQTS types) in European and American group of patients, But significantly higher in our population. We suppose different genetic factors could be chargeable: specific distribution of genetic forms, mutation spectrum or combination of pharmacogenetic variants. We have started molecular genetic studies of Iranian LQTS cohort to make these pointes clear.

Genomewide association analysis of metabolic phenotypes in a birth cohort from a founder population. *L. Peltonen[4,5,12], C. Sabatti[1,2], S. K. Service[3], T. Varilo[4,5], S. Gabriel[6], M. I. McCarthy[7], P. Elliott[8], M.-R. Jarvelin[8,9], N. B. Freimer[3,10,11]* 1) Dept of Human Genetics, UCLA, LA, California USA; 2) Dept of Statistics, UCLA, LA, California USA; 3) Center for Neurobehavioral Genetics, UCLA, LA, California USA; 4) Dept of Medical Genetics, U of Helsinki, Biomedicum Helsinki, Finland; 5) Inst of Molecular Medicine, Biomedicum Helsinki, Finland; 6) Broad Inst of Harvard and MIT, Cambridge, MA USA; 7) Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, UK; 8) Dept of Epidemiology and Public Health, Imperial College London, UK; 9) Inst of Health Sciences and Biocenter Oulu, U of Oulu, Finland; 10) The Jane and Terry Semel Inst for Neuroscience and Human Behavior, UCLA, LA, California USA; 11) Dept of Psychiatry, UCLA, LA, California USA; 12) Wellcome Trust Sanger Inst, Cambridge, UK.

Joint actions of genetic variants and environmental exposures determine the distribution of common diseases and traits within a population. Genome wide association studies (GWAS) offer an opportunity to dissect the complex etiology and epidemiology of such phenotypes, given a study sample appropriate for this purpose. We report here the first GWAS of a longitudinal birth cohort, Northern Finland Birth Cohort 1966 (NFBC1966) ascertained from the homogeneous late settlement region of Finland, for nine quantitative traits: triglycerides, high and low density lipoprotein, glucose, insulin, C-reactive protein, body mass index, and systolic/diastolic blood pressure in 4763 individuals from NFBC1966. We replicated most of 42 previously reported loci for these traits with genome wide significance and identified nine novel loci: a TG locus on 15q, HDL loci on 11p and 17p, LDL loci on 1q,11q and X, Glucose loci on 7q and 11q, and an Insulin locus on 10q. Genes highlighted encode proteins directly or indirectly associated with metabolism. In total, the identified genes explain on average about 5% of the total variability of the traits. The power of a founder population for detection of infrequent high impact alleles is demonstrated by identification of such an allele, in a novel LDL regulating gene on X, associated with dramatically increased LDL in males.

Is there a reason to study genes responsible for Joubert syndrome (JBTS) or Senior Loken syndrome (SNLS) in patients affected with isolated Leber congenital amaurosis (LCA)? *I. Perrault, N. Delphin, S. Hanein, S. Gerber, A. Munnich, J. Kaplan, J.-M. Rozet* Department of Genetics - INSERM U781 - Faculté Paris-Descartes, Paris, France.

Introduction: LCA is a genetically heterogeneous disorder characterized by a severe loss of vision in the first months of life. It is usually an isolated disorder but it can be associated with systemic features especially renal dysfunction (SNLS) or neurological symptoms and developmental delay (JBTS). Recently, mutations in the CEP290/NPHP6 gene have been shown to account for either isolated LCA or SLNS or JBTS. **Purpose:** The aim of the present study was to determine whether other genes responsible for syndromic LCA could account for isolated forms. **Families and Methods:** Thus, the NPHP4, NPHP5, NPHP7, NPHP8 and AHI1 genes were screened for mutations in 96 LCA cases unrelated to the 11 LCA genes. **Results:** No mutation was found in the NPHP4, NPHP7 and NPHP8 genes. Conversely, mutations were found in the NPHP5 (n=2/96) and AHI1 (n=1/96)genes, respectively. Both patients with NPHP5 mutations harboured two mutations expected to be severe. The natural history of the disease in these two young patients was reviewed and updated. At the age of 8yrs one of them developed a renal dysfunction compatible with the diagnosis of nephronophtisis. With regard to the second one no extraocular exam was available. The patient is under clinical investigation. The patient related to the AHI1 gene was found to carry a nonsense homozygous mutation. This girl aged of 27 months was unable to walk and presented with poor balance. Although MRI was still unavailable, JBTS is highly probable. **Conclusion:** this study suggests that genes responsible for SLNS and JBTS may not account for isolated LCA. Nevertheless, since the DNA of patients is generally sent soon after birth, the screening of SNLS or JBTS genes should be proposed in LCA infants to allow a close follow-up of those carrying mutations.

Refinement of genotype-phenotype correlation in the non-lissencephalic 17p13.3 contiguous gene deletion syndrome. *C. T. Thiel, C. Zweier, K. Hofmann, J. Hoyer, U. Trautmann, A. B. Ekici, A. Reis, A. Rauch* Institute of Human Genetics, University of Erlangen-Nuremberg, Erlangen, Germany.

Various heterozygous deletions in human chromosome 17p13.3-pter have been recognized to underlie the Miller-Dieker syndrome [MDS, MIM 24720], which is characterized by classical lissencephaly distinct facial dysmorphism and variable further features such as short stature and malformations of the heart and other organs. Classical lissencephaly occurs also as isolated brain malformation [ILS, MIM 607432] and is usually associated with severe mental retardation, intractable epilepsy and spasticity. ILS was shown to be caused by point mutations or deletions of the *PAFAH1B1* gene (*LIS1*) which is located within the MDS region in 17p13.3. Since the ILS deletion-region encompasses the *LIS1* and further telomeric genes but never includes *CRK* and *YWHAE*, a MDS critical region including these two genes was proposed, while haploinsufficiency of the further telomeric region was thought to be clinically insignificant. Recently, the more severe brain phenotype of MDS was explained by the additional deletion of the *YWHAE* gene interacting with LIS1 and NUDEL during brain formation. By molecular karyotyping we identified three novel non-lissencephalic patients with different submicroscopic deletions of the terminal 17p13.3 region allowing further refinement of the 17p- contiguous gene syndrome. Patient 1 showed a terminal 2.2 Mb deletion covering the MDS critical region but not *LIS1* and had normal brain MRI, mild to moderate mental retardation, the typical facial characteristics of MDS, short stature and TOF. Patient 2 had an interstitial 1.3 Mb deletion also covering the MDS critical region but not LIS1 and showed also mild to moderate mental retardation, MDS facial features, short stature, PDA and patent foramen ovale. A terminal 1.2 Mb deletion was identified in patient 3 with learning difficulties and mild facial anomalies, only. Our data confirm association of the MDS facial dysmorphism, mental retardation, short stature and congenital heart defects to the MDS critical region and exclude deletions of *YWHAE* without deletion of *LIS1* as causative of brain malformations.

CYTOGENETIC ANALYSIS OF A PATIENT WITH BLEPHAROPHIMOSIS PTOSIS EPICANTHUS INVERSUS SYNDROME (BPES). *P. Gupta[1], M. Kumar[2], M. Tanwar[2], N. Puskar[1], R. Dada[2]* 1) Dr. R.P. Centre for Ophthalmic Sciences, All India Institute Medical Sciences; 2) Lab. for Molecular Reproduction & Genetics, Deptt. of Anatomy, All India Institute Medical Sciences, New Delhi, India.

Blepharophimosis ptosis epicanthus inversus syndrome (BPES) is a rare genetic disorder with autosomal dominant pattern of inheritance. The frequency of BPES has been estimated to be 1 in 50,000. Patients with BPES have a combination of congenital anomalies as small palpebral fissures, epicanthus inversus, low nasal bridge and congenital ptosis. Other features like microphthalmos, anophthalamos, microcornea, hypermetropia, divergent strabismus, high arched palate, cup shaped ears, mental retardation and infertility in females has been reported. Based on the presence and absence of premature ovarian failure BPES has been categorized into two types: type I with infertility in females and type II involves eye malformation in both males and females. It has been shown that penetrance is 100% in type I where there is transmission by males only and 96.5% in type II in which transmission occurs through both sexes. From the review of reported cases, it has been concluded that a locus for eyelid development is situated at the interface of long arm of chromosome 3. Since blepharophimosis, ptosis and microphthalmia are consistent features in Patients with BPES an interstitial deletion of band 3q2, the location of BPES gene at this position seems highly likely. Various reports linked the deletion in 3q21, 3q22, 3q23, 3q24, 3q25 and translocations t(3:7)(q26-qter:q+), t(X:3)(p22:q21), t(3:8)(q23:p22.1) to the BPES. Thus cytogenetically different deletions and translocations of chromosome 3 have been described in patients with BPES. We report a sporadic case of BPES. We have done the Cytogenetic analysis of the patient. Standard GTG banding showed a novel deletion of band 3q26-28. This is the first report of deletion in this region. We also found deletion in the 3qter region which has been already linked to the BPES. This finding represents a severe manifestation of the disease. BPES is a heterogeneous entity, and evaluation and counseling of affected individuals should be undertaken with caution.

A novel mutation in the 3 UTR of SPG4 gene identified in an apparently sporadic patient affected by spastic paraplegia. *A. Magariello[1], L. Citrigno[1,2], A. Patitucci[1], R. Mazzei[1], F. L. Conforti[1], A. L. Gabriele[1], T. Sprovieri[1], C. Ungaro[1,2], M. Muglia[1]* 1) Inst Neurological Sci, National Research Council, Mangone, Cosenza, Italy; 2) Department of Neurosciences, Psychiatric and Anesthesiological Sciences, University of Messina, Messina, Italy.

Mutations in the SPG4 gene are the commonest cause of spastic paraplegia accounting for up to 40% of hereditary autosomal dominant and 12% of sporadic cases. The phenotype associated with disease due to mutations in the SPG4 gene tends to be pure. There is increasing evidence, however, of patients with complex forms of spastic paraplegia in which spastin mutations were identified. More than 150 point mutations have been identified in SPG4 gene and recently partial deletions of the gene were found in a significant number SPG4 point mutation negative patients. A 42 years old male with an apparently sporadic pure form of spastic paraplegia was referred for molecular diagnosis of SPG4 mutations. A novel point mutation, c. *2G>T, was identified in the 3UTR of the gene after two nucleotide from stop codon in exon 17. The mutation was not found in 200 normal chromosomes analyzed from the same geographical area. The web splice-site prediction software (http://www.fruitfly.org) revealed that the transition G>T creates a novel donor splice-site producing an alternative splicing involving the 3UTR region highly conserved and necessary to RNA stability. To predict the influence of this mutation, an RT-PCR reaction using RNA from the proband and from the normal control was carried out. Direct sequence of cDNA amplicon spanning from the exon 14 to 324 nts downstream of the termination codon revealed the presence of the wilde type but not the mutant allele thereby demonstrating skipping of 3UTR gene. In conclusion, the novel point mutation, c. *2G>T, here reported is the first pathological mutation identified in the 3 UTR of the SPG4 gene. ACKNOWLEDGEMENTS This work was partly supported by an Italian Ministry of Health grant (RF Neuro ex art56) to M.M.

Association of SNP (G-A) in the exon-1 of CRYAA gene with age related cataracts. *S. BhagyaLaxmi[1], T. Padma[1], R. Sireesha[1], M. Mamata[1], V. Dhananjay Raje[2], k. Sandhya[2], K. Ravi Kumar Reddy[3], M. Mundada[3]* 1) Department of Genetics, Osmania University, India; 2) Ocimum Biotechnologies, India; 3) Sarojini Devi Eye Hospital and Institute of Opthalmology,India.

455 age-related cataract patients (Nuclear (NC)-108; Cortical (CC)-105; Posterior subcapsular (PSC)-96; Mixed type (MT)-146 cataracts) and 144-age-matched controls were screened for SNPs in CRYAA-Ex-1 by SSCP and subsequently by sequencing. Demographic analysis showed high predilection of female (54.7%) as compared to male patients (45.2%). The mean ages-at-onset of cataracts were 59.80.92 in NC; 57.0201.1 in CC; 54.51.22 in PCS and 57.40.80 in MT. A high incidence of presenile (age-at-onset 50 years) cases were observed in PSC (34.0%) and CC (23.0%) as compared to NC (6.4%). The frequency of smokers was high in NC (53.0%) compared to CC (47.0%); PSC (38.0%) and controls (37.0%). Familial incidence was high in CC (7.6%) as compared to NC (5.6%) and PSC (3.1%). Screening of SNP (G-A) transition in the first exon-1 of CRYAA showed significantly high frequency of GA as compared to GG and AA patterns in all the patients in general and specifically in NC and CC cases (controls-17.0%, total cases -27.0%, NC -33.0% CC -29.0%; chi-square values: Total cases-2-5.87,p0.01; NC-2-9.45,p0.005; CC-2-5.06,p0.005).This suggests high risk for GA carriers for developing NC and CC cataracts. When the data was stratified, GG types showed a significant association when familial and non-familial cases (2-5.39,p0.02) were compared. Smokers and alcoholic patients of NC also showed significant association with GG genotypes as compared to controls (smokers- 2-5.77,p0.01; alcoholic- 2-8.36,p0.01). Alcoholic patients of CC showed a significant association with heterozygotes (GA-2-4.9;p0.02) as compared to other. The present results reveal difference in the association of the SNP (G-A) in exon-1 of CRYAA gene with reference to the type of cataract suggesting that this SNP may be used as a marker in predicting the risk for NC and CC types. Further this SNP may be used to follow up these cases (NC and CC) to determine the effect of pharmacotherapy used and modify treatment regimen accordingly.

Effects of extreme vs. severe hyperhomocysteinemia in mice. *S. Gupta[1], M. Slifker[1], J. Kühnisch[2], S. Lhoták[3], R. Austin[3], W. Kruger[1]* 1) Division of Population Science, Fox Chase Cancer Center, Philadelphia, PA; 2) Institute for Medical Genetics, Charitè, Universitätsmedizin, Berlin, Germany; 3) Henderson Research Centre, Hamilton, Ontario, Canada.

Cystathionine beta-synthase (CBS) deficiency in humans cause extreme elevations in plasma total homocysteine (tHcy; 200 M) resulting in vascular, skeletal, neurological, and visual phenotypes. Thrombosis is the main cause of morbidity in CBS deficient patients. Treatment with B-vitamins, betaine, and methionine restriction leads to a dramatic reduction in thrombotic events despite the patients often still having severe elevations in tHcy (80 M). To understand the difference between extreme and severe hyperhomocysteinemia we have studied two different mouse models of CBS deficiency. TgI278T Cbs-/- mice have extreme hyperhomocysteinemia with a mean tHcy of 296 M, while TghCBS Cbs-/- have severe hyperhomocysteinemia with a mean tHcy of 170 M. TgI278T Cbs-/- mice, but not TghCBS Cbs-/- mice showed osteoporosis of femur and fifth lumbar vertebrae, facial alopecia and a 20% reduction in mean survival compared to control mice. Metabolic profiling of serum reveals that TgI278T Cbs-/- mice have significantly elevated levels of homocystine, homocysteine-cysteine and homocysteine-glutathione disulfides but not protein bound homocysteine (bHcy) compared to TghCBS Cbs-/- mice. TgI278T Cbs-/- mice also showed elevated serum methionine, threonine, serine, glutamine, alanine, citrulline, ornithine and proline, and reduced cystathionine and total cysteine compared to TghCBS Cbs-/- mice. In the liver, TgI278T mice have significantly elevated Hcy, tHcy, bHcy, methionine, serine, glutamic acid, ornithine and AdoHcy in comparison to TghCBS mice. Livers from TgI278T but not TghCBS Cbs-/- mice showed ER stress as examined by immunohistochemical staining for ER stress markers and by qRT-PCR. Microarray studies on liver from TgI278T Cbs-/-, TghCBS Cbs-/-, and control mice indicate that the TgI278T Cbs-/- and TghCBS Cbs-/- mice have unique expression profiles. Our results indicate that there is a clear threshold effect in tHcy that distinguishes the phenotypic effects of extreme from severe hyperhomocysteinemia.

Absence of a functional Aurora Kinase C protein causes meiosis I arrest in men and leads to the production of large-headed multiflagellar spermatozoa. *K. Dieterich[1,2], R. Zouari[3], M. C. Jacob[4], M. Kharouf[3], J. Lunardi[1,2], S. Hennebicq[1,2,5], P. F. Ray[1,2]* 1) Génétique et Procréation, CHU de Grenoble, Grenoble, France; 2) Faculté de Médecine-Pharmacie, Domaine de la Merci, Université Joseph Fourrier, France; 3) Centre de FIV les jasmins, Tunis, Tunisia; 4) Laboratoire d'Immunologie Cellulaire, EFS Rhône Alpes, site de Grenoble. France; 5) Inserm U823, Institut Albert Bonniot, Grenoble, France.

We have recently identified a point mutation in the Aurora Kinase C (AURKC) gene (1) in infertile North African men presenting with oligoasthenoteratozoospermia (OAT) and typical large-headed multiple-tailed spermatozoa (OMIM 243060). This form of male infertility had been linked to a defect in chromosome/chromatid segregation during meiosis I and II and cytokinesis failure as suggested by the detection of 100% chromosomal abnormalities in the spermatozoa by FISH. We carried out a whole genome scan in 14 infertile men and could identify a homozygous cytosine deletion (c.144delC) in AURKC exon 3 of all fourteen patients. This frameshift mutation yields a premature stop codon at position 71 and thus a truncated protein with a reduced kinase domain. In the light of the recent understanding of the physiological and pathological role of Aurora Kinase C, large-headed multiple-tailed spermatozoa in men are expected to be tetraploid, yet FISH analyses showed a very heterogeneous ploidy pattern within the analysed spermatozoa with only a minority of tetraploid cells. We therefore developed a DNA flow cytometry assay to directly measure the quantity of DNA present in large-headed multiple-tailed spermatozoa. Our results, obtained on 5 index cases, demonstrate that all of these patients spermatozoa are indeed tetraploid. We conclude that the absence of a functional AURKC protein yields a blockage at meiosis I in spermatogenesis. These data highlight the limitation of the FISH technique, particularly in hyperhaploid cells. No haploid gametes were present in these patients sperm therefore formally contraindicating intracytoplasmic sperm injection (ICSI). 1) Dieterich et al. (2007) Nat Genet 39, 661-665.

Textile Plot: a Graphical Representaion for Multiple SNP Data. *N. Kumasaka[1], N. Kamatani[1,2]* 1) Center for Genomic Medicine, RIKEN, Tokyo, Japan; 2) Institute of Rheumatology, Tokyo Women's Medical University, Tokyo, Japan.

The textile plot (Kumasaka and Shibata 2008) is a graphical representation for very high dimensional data. It can accommodate not only numerical data but also unordered or ordered categorical data, or the mixture of those with missing values. The advantages of the textile plot in the context of association studies are presented with several practical examples. On the textile plot, a genotype at each SNP locus is represented by a point on a parallel coordinate axis, and a pair of genotypes between adjacent loci are connected by a segment with a width proportional to the number of replicates associated with the pair of genotypes. The locations and scales of all genotypes are appropriately determined so that all connected segments are aligned as horizontally as possible. From genetics point of view, the horizontalness incorporated into the textile plot represents a degree of non-independency within a population of a gamete's alleles at different loci. The plot can accentuate the gametic phase disequilibrium by specific geometrical shapes. In the context of maximum likelihood estimation, the absolute linkage disequilibrium (r^2=D'=1) between two loci is represented by all connected segments horizontally aligned, and the complete linkage disequilibrium ($r^2$1, D'=1) is represented by connected segments without line crossing on the textile plot. On the other hand, the population stratification within the given data can also be observed by vertical positions of genotypes on axes. As a result, the textile plot suggests several potential avenues of association studies to reveal significant population structure within the multiple SNP data which helps to detect disease susceptibility loci in the human genome.

Assessing the combined impact of 18 common genetic variants of modest effect sizes on type 2 diabetes risk. *M. N. Weedon[1], H. Lango[1], C. N. A. Palmer[2], A. D. Morris[2], E. Zeggini[3], A. T. Hattersley[1], M. I. McCarthy[3], T. M. Frayling[1]*
1) Peninsula College of Medicine and Dentistry, Exeter, UK; 2) Ninewells Hospital and Medical School, University of Dundee, UK; 3) Wellcome Trust Centre for Human Genetics, University of Oxford, UK.

Genome-wide association studies have dramatically increased the number of common genetic variants that are robustly associated with type 2 diabetes (T2D). A possible clinical use of this information is to identify individuals at high risk of developing the disease, so that preventative measures may be more effectively targeted. Here we assess the ability of 18 confirmed T2D variants to differentiate between T2D cases and controls.

We assessed index SNPs for the 18 independent loci in 2598 controls and 2309 cases from the GoDARTS study, a population-based case-control cohort. The discriminatory ability of the combined SNP information was assessed by grouping individuals based on number of risk alleles carried and determining relative odds of T2D, and by calculating the area-under the receiver-operator characteristic curve (AUC).

Individuals carrying more risk alleles had higher risk of T2D. For example, 1.2% of individuals with >24 risk alleles had an odds ratio of 4.2 (95% CI: 2.11, 8.56) against the 1.8% with 10-12 risk alleles. The AUC (a measure of discriminative accuracy) for these variants was 0.60. The AUC for age, BMI and gender was 0.78, and adding the genetic risk variants only marginally increased this to 0.80.

Currently, common risk variants for T2D do not provide strong predictive value at a population level. However, the joint effect of risk variants identified sub-groups of the population at substantially different risk of disease. Further studies are needed to assess whether individuals with extreme numbers of risk alleles may benefit from genetic testing.

Whole genome expression analysis to study the role of severe and mild GBA mutations in Parkinson's disease. *A. Orr-Urtreger[1,3], M. Kedmi[1], A. Bar-Shira[1], N. Giladi[2,3], Z. Gan-Or[1,3]* 1) Genetics Institute and; 2) Dept Neurology, Tel Aviv Sourasky Med Ctr; 3) Sackler Faculty of Medicine, Tel Aviv Univ, Israel.

Association between GBA (-glucocerebrosidase) gene mutations and Parkinson's disease (PD) was established. The high carrier frequency of GBA mutations among Ashkenazim (1/16) renders this population important for studying GBA role in PD. Rrecently, we demonstarated a genotype-phenotype correlation between mild and severe GBA mutations and PD risk and age at motor symptoms onset (AAO) (Neurology 2008). This association is further analyzed here in an extended cohort of 600 patients and 353 elderly controls. Additionally, to detect genes that might be involved in GBA-associated PD, we initiated a global gene expression analysis of peripheral blood leukocytes (PBLs) samples from patients carrying mild and severe GBA mutations and controls. Gene expression and alternative splicing profiles were analyzed by Affymetrix GeneChip Human Exon 1.0 ST Arrays. The frequency of GBA carriers was 19.3% in PD patients compared to 4.0% in the elderly controls ($p<0.01$). The proportion of severe mutation carriers among PD patients-GBA carriers was 24% compared to 7% among average risk controls ($p<0.01$). Severe and mild GBA mutations significantly increased the risk for developing PD by 10.9 and 2.7-fold, and significantly affected the AAO of PD, 55.2 and 58.7 years, compared to 61.0 years in patients without known GBA or LRRK2 mutations. Analysis of the "core" exon-expression data (21,980 genes and 232,448 exons) revealed a differential expression of 921 and 135 genes ($p<0.05$ and $p<0.01$, ANOVA), when comparing PBLs from severe and mild GBA mutations carriers, and differential splicing of 135 genes (bonferroni corrected $p<0.05$, Alt-Splice ANOVA). Our results demonstrate the differential effects of severe versus mild GBA mutations on PD risk and AAO, and reveal different profiles of genes expression and alternative splicing in these two genotypic groups of patients. They also emphasize the uniqueness of our population for studying the complex genetic basis of PD, and suggest that PBLs may serve as a relevant surrogate tissue to examine the transcriptional changes involved in PD pathogenesis.

Genetic variants of -chemokines are associated with autoimmunity in humans and rats. *J. Öckinger[1], M. Jagodic[1], F. Lundberg[1], I. Kockum[1], L. Padyukov[2], E. Wallström[1], J. Hillert[1], T. Olsson[1]* 1) Clinical Neuroscience, Karolinska Institutet, Stockholm, Stockholm, Sweden; 2) Department of Medicine, Karolinska Institutet, Sweden.

Identification of genetic factors that regulate autoimmune disorders, such as multiple sclerosis (MS), is crucial for unraveling the disease mechanisms. In this study we have utilized a rat model of MS; myelin oligodendrocyte glycoprotein induced experimental autoimmune encephalomyelitis (MOG-EAE), in order to identify genetic regions that regulate rodent neuroinflammation. Linkage analysis in an advanced intercross line (AIL) originating from the DA and PVG.AV1 rat strains identified *Eae18b*, a 1.06Mb region on rat chromosome 10 that regulates severity of MOG-EAE. *Eae18b* includes a cluster of -chemokines, a class of molecules with immunostimulatory and chemoattractant properties.

In addition to the genetic studies in the rat, we investigated the human homologues of the -chemokines in an association study. In total 18 SNPs in 6 chemokine genes were analyzed in 1079 patients diagnosed with MS and 1198 control individuals. The results show that genetic variants of the CCL8 gene are associated an increased risk for MS, whereas haplotypes including the CCL13 and CCL1 genes are protective. Selected SNPs were also investigated in 2158 patients diagnosed with rheumatoid arthritis (RA) and 1068 control individuals. We could thereby show that genetic variants in the CCL2 gene are associated with an increased risk for RA.

The results from this study imply that genetic variants of the chemokines regulate neuroinflammation in both rats and humans, as well as other inflammatory diseases in humans.

Identification of CFTR rearrangements by a CGH locus specific array. *C. Le Marechal, S. Quemeneur, C. Benech, K. Giteau, MP. Audrezet, C. Ferec* Inserm U613,University-hospital,Brest ,France.

More than 1500 CFTR mutations have been described and deposited in the cystic fibrosis mutation data base(www.genet.sickkids.on.ca/cftr) so far .In the few past years, we(Audrezet et al ;Ferec et al) as well as others (Niels et al,Handash et al,Chevalier-Prost et al,) have developed new semi quantitative approaches to evidence gross rearrangements in this gene .We have shown that these deletions account for about 1 to 3% of the CF disease causing mutations worldwide. We have described 8 new rearrangements and characterized their breakpoint junctions .In this work we have designed a 15K locus specific CFTR array (Agilent technology) to carefully analyze the deletions/insertions or duplications at this locus. We showed that the 17 deletions /insertions collected in our lab from different countries worldwide are clearly evidenced and moreover the 5 and 3breakpoint of these deletions are accurately determined.. This allows a rapid design of primers to amplify the breakpoint junction and to obtain the sequence permitting the characterisation of the defect at the molecular level. The CGH locus specific 7q3.1 array that we have developed is an excellent new tool allowing a rapid scan and characterization of the rearrangements that could affect not only the 27 exons of the gene but also those that could be located throughout the 200 kb genomic region of the CFTR gene. Audrezet MP, et al,Hum Mut,2003, 23:343-357 Ferec C,et al ,Eur J Hum Genet,2006,14:567-576 Niels F,et al,J Med Genet 41:e118 Handash et al ,Human mut,2005,25:504 Chevalier-Prost F,Human Mut,2005,25:504-509.

AsiDesigner: exon-based siRNA design server considering alternative splicing. *Y. Kim, Y. Park, M. Won* Functional Genome Research Ctr, KRIBB, Daejeon, Korea.

RNA interference (RNAi) with small interfering RNA (siRNA) has become a powerful tool in functional and medical genomic research through directed post-transcriptional gene silencing. In order to apply RNAi technique for eukaryotic organisms, where frequent alternative splicing results in diversification of mRNAs and finally of proteins, we need spliced mRNA isoform silencing to study the function of individual proteins. AsiDesigner is a web based siRNA design software system which provides siRNA design capability to account for alternative splicing for mRNA level gene silencing. It provides numerous novel functions including the designing of common siRNAs for the silencing of more than two mRNAs simultaneously, a scoring scheme to evaluate the performance of designed siRNAs by adopting state-of-the-art design factors, a stepwise off-target searching with BLAST and FASTA algorithms and checking the folding secondary structure energy of siRNAs. To do this, we developed a novel algorithm to evaluate the common target region where siRNAs can be designed to knock down a specific mRNA isoform or more than two mRNA isoforms from a target gene simultaneously. The developed algorithm and the AsiDesigner were tested and validated as very effective throughout widely performed gene silencing experiments. It is expected that AsiDesigner will play an important role in functional genomics, drug discovery, and other molecular biological research. AsiDesigner is freely accessible at http://sysbio.kribb.re.kr:8080/AsiDesigner/.

Impact of definitive haplotypes on genetical analyses. *K. Higasa, Y. Kukita, T. Tahira, K. Hayashi* Res Ctr Gen Info, Kyushu Univ/Med Inst Bioreg, Fukuoka, Japan.

The haplotype map constructed by the International HapMap Project is a valuable source for the studies of disease genes, population structure, and evolution. In the Project, haplotypes have been inferred from experimentally determined genotypes, and are fairly accurate for Caucasians and Africans since the inference was based on the genotypes of trios. However, the inference for the Asians populations was less accurate, because of the lack of familial information. Here we assessed how the error in the inference can affect downstream studies, especially the analysis of recent positive selections, by comparing the results of the analyses using the data of HapMap JPT and of definitive haplotypes (DHaplo-DB) determined by us from a collection of Japanese complete hydatidiform moles (CHM), each of which carries a genome derived from a single sperm. We found that the error in JPT was not uniform throughout the genome, and the statistics for recent positive selection was significantly affected. The most pronounced region was MHC locus, where the signal of positive selection was not detected for JPT, when analyzed using haplotype data determined without family information, but detected when analyzed using definitive haplotype data. The long-range LD and conservation of long haplotypes may have rendered this locus particularly sensitive to phasing error. The D-HaploDB is freely accessible via the internet at http://finch.gen.kyushu-u.ac.jp.

Preprorenin signal peptide mutation in a family with uromodulin-associated kidney disease. *M. Zivna[1], K. Hodanova[1], P. Vyletal[1], J. Sikora[1], V. Baresova[1], H. Hulkova[1], M. Kalbacova[1], J. Zivny[2], R. Ivanek[1,3], K. Kapp[4], M. Elleder[1], S. Kmoch[1]* 1) Inst of Inherit Metabol Dis, Charles University, Prague; 2) Dept of Pathophysiology, Charles University, Prague; 3) Inst of Mol Genet, Academy of Science, Prague; 4) Center for Mol Biol, University of Heidelberg.

The term of uromodulin-associated kidney diseases (UAKD) emerged recently for a group of autosomal-dominant tubulointerstitial nephropathies characterized by combination of hyperuricemia, gouty arthritis, decreased urinary uromodulin (UMOD) excretion and abnormal UMOD expression in the kidney. UAKD comprise phenotypes known as a familial juvenile hyperuricemic nephropathy and medullary cystic kidney diseases type 1 and type 2. The association of these phenotypes with UMOD changes was established following the identification of disease causing mutations in the UMOD gene in most of the affected families. In a single family with no UMOD mutation we used positional cloning and identified in patients unique deletion of one of a leucine residues forming hydrophobic penta-leucin motif of the preprorenin signal peptide (L16REN). To characterize pathogenic effect of the identified mutation we performed in-vitro translation/translocation assays, expressed WTREN and L16REN in ATt-20 and HEK293 cell lines, characterized qualitative and quantitative parameters as well as cellular localization of the recombinant proteins, assessed effect of the L16REN mutation on cell viability and correlated the results with expression of prorenin and renin, other phenotype related proteins and selected tubular markers in available patient kidney. Our results have provided several lines of evidence indicating that the identified mutation causes aberrant cotranslational translocation of preprorenin, reduced rate of prorenin and renin biosynthesis and abnormal localization of prorenin and renin in patient kidney. We hypothesize that, as in renal tubular dysgenesis and animal models with renin dysfunction, the observed abnormalities may initiate endoplasmic reticulum stress and slowly progressing damage of juxtaglomerular cells, which may end up in deterioration of kidney functions and observed clinical abnormalities.

Interplay of genetic and lifestyle influences on myocardial infarction in South Asia: The Pakistan Risk of Myocardial Infarction Study (PROMIS). *P. Frossard[1], D. Saleheen[1,2], J. Danesh[2]* 1) Dept Biological & Biomed Sci, Aga Khan Univ Med Col, FHS, Karachi, Pakistan; 2) Department of Public Health and Primary Care, University of Cambridge, UK.

The Pakistan Risk of Myocardial Infarction Study (PROMIS) aims at providing a resource for the assessment of the interplay of genetic and lifestyle influences on coronary heart disease (CHD) in Pakistani South Asians. A total of 10,000 individuals aged 30-80 years (both male and female) with a first-ever confirmed myocardial infarction (MI) and 10,000 frequency-matched controls (on age and sex) is being recruited with complete medical, social, and dietary information. Serum, plasma, whole blood and extracted DNA samples are taken from each participant for biochemical and genetic analyses, and cell-lines are maintained for studies of gene expression. Preliminary data on 3000 MI cases and 3000 controls indicates that the mean age of first MI cases is 54 (SD 11) years; the prevalence of diabetes (16% among cases, 10% among controls) and the use of tobacco products (51% among cases, 41% among controls) is substantially higher than in Western populations; levels of HDL-C, however, are substantially lower (0.81 mmol/L in cases and 0.86 mmol/L in controls). This suggests that reverse cholesterol transport and metabolic dysfunction could be of particular relevance to MI in South Asians. Recent genome-wide investigations on subjects of European descent reported that two novel loci (rs599839 and rs646776) at chromosome 1p13.3 are strongly associated with reduced LDL-C levels. The first 2,806 participants of PROMIS were genotyped for rs599839 and rs646776 using a TaqMan assay. The two variants display a high level of linkage disequilibrium (R2 = 0.96) and were associated with 0.13 mmol/L and 0.15 mmol/L reduction in LDL-C levels, and with 28% and 29% reduced risks of MI, respectively, thereby confirming and validating the original findings in the PROMIS population. Establishing a large bioresource, as proposed in PROMIS, identifies the determinants of CHD in South Asia and will contribute to locally appropriate strategies to prevent and control the growing epidemic of CHD in this region.

High density oligonucleotide array identifies a submicroscopic 3.2 MB chromosomal 16q12.2 deletion in a child with Noonan-like phenotype. *C. F. Chang[1,2], A. Tsai[4], L. H. Li[1], J. Y. Wu[1], Y. T. Chen[1], F. J. Tsai[3]* 1) IBMS Academia Sinica,; 2) Molecular Medicine Program, IMI, National Yang Ming University,; 3) Department of Medical Genetics & Medical Research, China Medical University Hospital,Taiwn; 4) Div Clinical Gen & Metabolism, Children Hosp, Denver, CO.

Chromosomal aberrations are a common cause of multiple anomalies, developmental delay and dysmorphic features. Array based comparative genomic hybridization (array-CGH) that covers the whole genome can pick up rearrangement not previously detected by conventional G-banding or FISH for specific/critical regions. We report a 9- year-old boy with Noonan-like facial features without congenital heart disease. Other features include short stature and mild mental retardation. Physical exanimations were significant for broad forehead, hypertelorism, flat nasal bridge, down-turn mouth corners, high arch palate, cup-shaped/over-folded auricles, left preauricular tag, low set and posteriorly rotated ears, short neck, single palmar crease, and prominent gap between first and second toes. Karyotype and FISH for 22 were normal. Using high density oligonucleotide chip Affymetrix Genome-Wide Human SNP Array 6.0, we identified a de novo 3.2 Mb deletion from bands q12.2 to q13 on chromosome 16 (from rs8044091 to rs4783954). The study was designed to perform parental and proband samples concurrently on three chips and interpreted as a trio set. The deletion was confirmed by qPCR of SLC6A2 and FTO genes which locate in the deleted region. Chromosomal regions harboring genes associated with Noonan or Noonan-like syndrome, such as 12q21.1, 12p12.1, 2p22, 3p25 and 17q11 showed no copy number changes. Our study demonstrated the power of high density oligonucleotide array chip in identifying novel deletions and delineating unreported phenotype. Our patient also suggests another critical region for Noonan-like facial features. In patients who have Noonan -like facial features with short stature without heart finding, high density array should be included in initial work up prior than sequencing analysis for RAS/MAP kinase pathway genes.

The association among the metabolic syndrome, ABCA1 haplotype and lipid metabolism. *C.-L. Lee[1], Y.-T. Lin[1], R.-Y. Wang[2], C.-Y. Chen[2], C.-J. Lin[3], M.-C. Humg[3], W.-C. Shy[3], T.-N. Wu[1], F.-Y. Wu[1]* 1) China Medical University, Environmental Health, Taichung, Taiwan; 2) China Medical University, Public Health, Taichung, Taiwan; 3) China Medical University, Nursing, Taichung, Taiwan.

The aim of this study was to investigate the relationships among the metabolic syndrome, haplotypes with single nucleotide polymorphisms (SNPs; G1051A, G2706A and A3044G) in the ATP-binding cassette transporter 1 (ABCA1) and lipid metabolism. Participants in the National Adult Health Examination Project in Year 2007, who were residents in the middle of Taiwan, were recruited as the study subjects. 276 subjects with metabolic syndrome (MetS) were selected. The remaining 406 subjects did not have the metabolic syndrome (non-MetS). In the MetS group, 27.2% had a BMI 27-30 and 27.2% had a BMI30. And in the non-MetS group, the rates for BMI 27-30 and BMI30 were 10.9% and 6.1%, respectively. Anthropometric measures and biochemical analytical results showed that subjects in the MetS group had significantly higher glucose, triglycerides, -GT, systolic pressure, diastolic pressure, waist circumference, and BMI. MetS group subjects also had lower HDL concentrations and had more comorbidities (such as heart disease, hypertension, obesity, hyperuricemia, blood lipid abnormalities, etc.). All of these above differences between the MetS and non-MetS groups were statistically significant. The results of multivariate logistic regression analysis indicated that subjects with chewing betel nut habit would have 3.46 times the risk of having metabolic syndrome than those without (95% CI=1.27-9.4). On the other hands, subjects with drinking coffee habit would decrease the risk of having metabolic syndrome (OR=0.31; 95% CI=0.12-0.76). After adjusting for the effects of age, gender, ethnicity and BMI), the haplotype with SNPs (1051A, 2706A, 3044G) in ABCA1 was associated with the risk of metabolic syndrome (OR=7.6, 95% CI=2.37-24.31). In conclusion, there were also a greater number of total comorbidities in MetS subjects than in non-MetS subjects. People carrying the haplotype with SNPs in ABCA1 were at a higher risk of having the metabolic syndrome.

Genetic Skeletal Dysplasias in Thailand: Twenty -year experience. *P. Wasant, N. Vattanawicharn, S. Liammongkolkul* Dept of Ped/Div of Medical Gen, Siriraj Hosp/Mahidol Univ, Bangkok, Thailand.

Genetic skeletal dysplasias are a heterogeneous group of disorders associated with abnormalities in the size and shape of the limbs , trunk , and/ or skull that frequenthy result in disproportionate short stature. There are well over 100 distinct skeletal dysplasias that have been classified primarily on the basis of their clinical or radiographic characteristics. The Genetic Skeletal Dysplasia Clinic was established at the Division of Medical Genetics, Department of Pediatrics, Faculty of Medicine Siriraj Hospital, Mahidol University in Bangkok since 1992, the only one of its kind in Thailand. Main objective of the clinic is to educate Thai pediatricians and orthopedic surgeons in training (from multicenter) about various genetic skeletal disorders; and how to care and treat pediatric patients with skeletal dysplasias appropriately. There are numerous cases: achondroplasia, hypochondroplasia, pseudoachondroplasia, atelosteogenesis, pyknodysostosis, spondylo -epiphyseal dysplasia congenita, spondylometaepiphyseal dysplasia, osteogenesis imperfecta, chondroectodermal dysplasia (Ellis- van- Creveld), cleidocranial dysostosis, thanatophoric dysplasia, rhizomelic chondrodysplasia punctata, trichorhinophalangeal syndrome, mucopolysaccharidoses, (Hurler,Hunter,Sanfillipo, Morquio, Maroteaux-Lamy,Sly), osteopetrosis, campomelic dysplasia, Stickler syndrome, Kniest dysplasia, nail-patella syndrome, diastrohic dysplasia, short-rib polydactyly syndrome, Larsen syndrome, multiple epiphyseal dysplasia, metaphyseal dysplasia and hypophosphatemic rickets. The diagnoses are established at clinical, radiological and biochemical level .There are limitations in confirmation at the molecular level in developing countries. Multidisciplinary approach and genetic counseling are provided.

Search for cryptic subtelomeric chromosome imbalances in prenatal diagnosis of fetal malformations. *A. Soler[1], J. Bruguera[2], C. Morales[1], N. Clusellas[1], I. Mademont[3], I. Madrigal[3], E. Margarit[1], A. Sánchez[1]* 1) Servei de Bioquímica i Genètica Molecular, Hospital Clínic, Barcelona, Spain; 2) Fundació Clínic per a la Recerca Biomèdica, Barcelona, Spain; 3) CIBER de Enfermedades Raras (CIBERER), Barcelona, Spain.

The chromosomal abnormalities ascertained by conventional cytogenetics are responsible for part of the fetal malformations, but a majority of them show a normal karyotype. The presence of a cryptic imbalance may probably explain some of the abnormal phenotypes. MLPA (Multiplex Ligation-dependent Probe Amplification) has been developed as a reliable molecular technique to detect targeted submicroscopic imbalances. We selected 218 pregnancies presenting major fetal malformations (such as cardiopathies, skeletal abnormalities, etc), which had shown a normal conventional karyotype. MLPA using the p036 subtelomere kit (MRC-Holland) was performed in their corresponding amniotic fluid samples, and revealed 27 imbalances (12.4%). Confirmation studies were done using the complementary kit p070 (MRC-Holland); only 7/27 cases were confirmed (3.2%): deletion of 3pter, duplication of 13qter plus deletion of 20pter, duplication of 14qter, duplication of Ypter, duplication of 12qter and duplication of Xpter. FISH (Fluorescent in situ Hybridisation) was also performed to confirm some of the deletions. Discordant false positive cases are maybe due to different reasons. Some p036 imbalances have been previously reported as polymorphisms. Another reason is that p036 and p070 kits contain different probes and some imbalances are located very close to the telomere region so the more centromeric p070 kit does not confirm the results and FISH is not enough sensible to detect them. These discordant results need more accurate techniques for confirming some subtelomeric imbalances. Supported by the grant PI05/0096 to A.S. from Fondo de Investigaciones Sanitarias, Ministerio de Sanidad y Consumo, Spain.

Genetic study of SCN1A-related epilepsies in southern Italy. *E. V. De Marco[1], F. Annesi[1], G. Annesi[1], S. Carrideo[1], G. Provenzano[1], A. Labate[1,2], D. Civitelli[1], F. E. Rocca[1], L. Mumoli[2], E. Mannarino[1,3], G. Incorpora[4], G. Tortorella[3], A. Quattrone[1,2], A. Gambardella[1,2]* 1) Institute of Neurological Sciences, National Research Council, Cosenza, Italy; 2) Institute of Neurology, University Magna Graecia, Catanzaro, Italy; 3) Dept of Paediatrics, University of Catania, Italy; 4) Division of Infantile Neuropsychiatry, University Hospital of Messina, Italy.

The gene SCN1A has been found mutated in various types of epilepsy. Approximately 10% of generalized epilepsy with febrile seizures plus (GEFS+) families carry mutations in SCN1A. Furthermore, SCN1A mutations occur in more than 70% of patients with severe myoclonic epilepsy of infancy (SMEI) and are usually de novo. We have previously reported some novel SCN1A variants in our population. We aimed to extend the sample number to estimate the frequency of the SCN1A mutations in GEFS+ and SMEI patients in southern Italy. Twenty-two GEFS+ and twenty-six SMEI patients were recruited from Calabria and Sicily. Diagnoses were based on the ILAE criteria. All 26 exons of the SCN1A gene were sequenced. Among the 22 GEFS probands belonging to unrelated families, 3 (13.6%) had SCN1A variations that were previously published (Met1841Thr, Tyr779Cys, Ile1944Thr). Among the 30 SMEI patients, 5 (16.7%) carried SCN1A mutations. Four of them were already reported by ourselves (T1289I, 3840insT, IVS24-2A>G) and other authors (S1505X). A novel mutation was found in the splicing donor site of intron 7, consisting in a deletion of a single nucleotide (IVS7+4delA). SCN1A is the most clinically relevant epilepsy gene. More than 20 mutations have been associated to GEFS+ and nearly 200 mutations to SMEI. We found 8 patients carrying different mutations, thus confirming the high genetic heterogeneity related to these diseases. In our population we found a frequency of GEFS+ mutation similar to that reported by other authors, whereas the SCN1A variations are less represented in our SMEI patients in comparison to the overall literature. In spite of this, our results confirm that SCN1A mutations represent an important cause of GEFS+ and SMEI also in southern Italy.

ASSOCIATION STUDY BETWEEN HFE, TF, TFR1 GENES AND PARKINSONS DISEASE. *V. Greco[1], F. E. Rocca[1,2], F. Annesi[1], E. V. De Marco[1], G. Provenzano[1,3], D. Civitelli[1], P. Tarantino[1,3], V. Scornaienchi[1], G. Annesi[1]* 1) Inst Neurol Sci, National Research Council, Cosenza, Italy; 2) Institute of Neurology, University Magna Graecia, Catanzaro; 3) Departement of Neurosciences, Psychiatry and Anaesthesiology University of Messina, Messina Italy.

Iron overload increases oxidative stress and may lead to neurodegenerative disorders like Parkinson's disease (PD). Alterations of iron-related genes, therefore, might be involved in the pathogenesis of PD. The aim of this study is to investigate a possible association between the polymorphism C282Y of haemochromatosis (HFE) gene and the prevalence of PD in Southern Italy. Subsequently we will also analyze if the second common polymorphism H63D of the HFE gene, the polymorphisms G258S transferrin (TF) gene and S82G transferrin receptor1 (TFR1) gene are other genetic risk factors for PD. We analyzed the C282Y HFE polymorphism in 175 sporadic PD patients and in 175 controls from Southern Italy. The clinical diagnosis of PD was based according to the UK PD Society Brain Bank criteria. We carried out a genetic analysis by standard PCR and restriction digestion method. Among 175 patients screened for the HFE C282Y polymorphism, 3 (0.86%) carried heterozygous variation C282Y and we found 3 (0.86%) C282Y heterozygotes in the controls. The HFE protein is an important regulator of cellular iron homeostasis. Recent studies on the role of the HFE gene variants in PD vary from a protective effect of C282Y heterozygosity, no effect of the C282Y or H63D variant to an increased risk for PD. In a recent study on the Italian population, the HFE H63D and C282Y polymorphisms are not associated with PD. In Italy, the prevalence of C282Y is lower than in Northern European countries and it is also lower in Southern Italy than Northern Italy. Our results confirm the low frequency of HFE C282Y allele in Southern Italy. We havent found a different frequency between patients and controls, indicating no association between the C282Y HFE gene with PD. Furthermore we are still analyzing the polymorphisms H63D in the HFE gene, G258S in the TF gene and S82G in the TFR1 gene. Our study should be extended for the rarity of the HFE C282Y polymorphism.

GOLD (Gain or Loss Detection) Chip: a new array CGH tool for genetic diagnosis. *S. Comuzio[1], E. Ciabattoni[1], F. Motta[1], G. Stoico[1], E. Bartolini[1], G. Babbini[1], A. Novelli[3], G. Novelli[2], F. Gullotta[2], S. Gambardella[2]* 1) Technogenetics, Milan, Italy; 2) Department of Biopathology, Tor Vergata University, Rome, Italy; 3) Cytogenetic Service, Istituto Mendel-Casa Sollievo della Sofferenza, Rome, Italy.

We designed a new array CGH slide called GOLD (Gain or Loss Detection) Chip. The slide consist of 900 non-overlapping BAC (Bacterial Artificial Chromosome) clones spanning the whole genome, and accurately selected using public databases. The chip has a 10 Mb backbone to guarantee the detection of the aneuploidies, and has an implemented resolution for telomeres, and for regions involved in common genomic diseases. DNA was extracted from overnight bacterial cultures. Each DNA was amplified first by DOP PCR, then by amino PCR to allow the linking to the slides surface, and purified with Clean-up Kit. Each amino PCR was spotted random in six replicates, in two areas. Slides were incubated for 48 hours in a cross-linking box, then washed and blocked with 10 mg/ml BSA solution. Then the slides were validated with 45 DNA from cultured amniotic liquid and commercial cell lines, including the more common Trisomy (Klinefelter, Down, Edwards, Patau, Trisomy 9, Trisomy 22, Trisomy 16), Monosomy (Turner syndrome), and Microdeletions (Cat eye, Wolf-Hirschhorn syndrome, Cri du chat, Williams syndrome, Prader-Willi/ Angelman,Miller-Dieker, Smith-Magenis syndrome, 22q11.2 deletion syndrome). Experimental protocol consists of labeling 600 ng of DNA test and control, for dye-swap experiment, and hybridize 40 hours at 42 C in Hybridization Chambers. The slides were scanned at 5 micron and the images were analyzed using Bluefuse software (Bluegnome). Our protocol allowed to characterize all the DNA imbalances in the 45 samples analyzed. To encourage the use of GOLD Chip for both prenatal and postnatal diagnosis, we provide FISH probes correspondent to all the pathologies identifiable by our experimental protocol. For further informations visit www.technogenetics.it.

Impact of copy number variation (CNV) definition on a CNV association study of amyotrophic lateral sclerosis (ALS). *L. V. Wain[1], A. Al-Chalabi[2], P. N. Leigh[2], C. E. Shaw[2], R. H. Brown[3], J. E. Landers[3], M. D. Tobin[1]* 1) Departments of Health Sciences and Genetics, University of Leicester, UK; 2) MRC Centre for Neurodegeneration Research, Kings College London, Department of Clinical Neuroscience, Institute of Psychiatry, London, UK; 3) Cecil B. Day Laboratory for Neuromuscular Research, Massachusetts General Hospital East, Charlestown, MA, USA.

Copy number variation (CNV) is a major contributor to human genetic variation and CNV associations to disease are emerging. The development of CNV-specific microarrays is in its infancy but many studies have attempted, with varying success, to exploit existing SNP genotyping array data for use in CNV discovery and association studies. Many algorithms have been developed to screen such data for CNV but how to utilize these data in association studies still presents a major challenge. We analysed Illumina 317K SNP array data from 967 ALS patients and 1001 control individuals for CNV using a Hidden Markov Model method (QuantiSNP). Measures based on the overall and relative probe fluorescence intensities for each SNP are used to infer copy number variation across the genome. The method we used identifies putative CNVs, on a sample by sample basis, that overlap by varying degrees CNVs detected in other samples. Large overlapping or nested CNVs are known to have different clinical consequences in some genomic disorders, such as 17p11.2 deletion and duplication syndromes. A key issue in classifying CNVs or association studies is how to treat multiple overlapping hits at the same locus. Whilst analysis of sample and assay quality is essential to identify which samples should be included or excluded from the CNV detection analysis, careful downstream processing of the CNV calls is crucial to forming the basis of a good CNV association study. We explored how best to undertake this in order to retain as much information as possible for association testing whilst ensuring a low false positive rate. Our initial findings suggest that algorithms that simply combine overlapping calls may result in loss of information due to genuinely overlapping CNVs. Our results should help inform the analytical strategies for other CNV association studies.

The C allele of rs2237895 *KCNQ1* is associated with reduced pancreatic beta-cell function in middle-aged Danish people. *J. Holmkvist[1], K. Banasik[1], G. Andersen[1], H. Unoki[2], K. Færch[1], T. Jørgensen[3,5], K. Borch-Johnsen[1,3,4], S. Maeda[2], T. Hansen[1], O. Pedersen[1,4,5]* 1) Steno Diabetes Center, Gentofte, Denmark; 2) Laboratory for Endocrinology and Metabolism, Center for Genomic Medicine, RIKEN, Yokohama, Kanagawa, Japan; 3) Research Centre for Prevention and Health, Glostrup University Hospital, Glostrup, Denmark; 4) Faculty of Health Science, University of Aarhus, Aarhus, Denmark; 5) Faculty of Health Science, University of Copenhagen, Copenhagen, Denmark.

BACKGROUND AND AIM: The objective of this study was to investigate if polymorphisms in the recently identified type 2 diabetes (T2D) gene potassium channel, voltage-gated, KQT-like subfamily, member 1 (*KCNQ1*), found on chromosome 11p15.5, are associated to T2D-related quantitative traits. RESEARCH DESIGN AND METHODS: A polymorphism associated to T2D in a recent genome-wide association scan (rs2237895 [A/C]) was genotyped in 6,038 treatment-naïve subjects of the Danish population-based Inter99 cohort involving 4567 with normal glucose tolerance, 508 with impaired fasting glucose, 707 with impaired glucose tolerance and 256 with screen-detected T2D. A general linear model was used to test quantitative variables for differences between genotype groups of the examined subjects. RESULTS: The C-allele of rs2237895 was strongly associated with reduced post-OGTT measures of insulin release under a dominant genetic model: serum insulin 30min (meanSD: 286184 vs. 302196 pmol/l, p=0.0008), incremental area under the curve for insulin (2250715960 vs. 2367316564 pmolmin, p=0.006), serum C-peptide 30min (1986720 vs. 2033710 pmol/l, p=0.007), insulinogenic index (28.619.4 vs. 30.721.1, p=0.002) and disposition index (insulinogenic index / HOMA-IR) (3.62.8 vs. 3.83.0, p=0.01). Association with reduced measures of insulin release was predominantly driven by the female gender e.g., insulinogenic index (p_{female}=0.0004, p_{male}=0.22). CONCLUSION: The C-allele of rs2237895 in *KCNQ1* is associated with reduced measures of insulin release, a reduction that is significantly more pronounced in women compared to men.

Modeling Aberrant Splicing in Mutant Genes associated with Inherited Retinal Degeneration and Bardet-Biedl Syndrome. *C. E. Willoughby, D. O'Prey, D. A. C. Simpson* Centre for Vision Science, Queen's Univ Belfast, Belfast, United Kingdom.

Up to 50% of all point mutations responsible for genetic diseases cause aberrant splicing. The aim of this study was to model the pathological impact of known mutations on the splicing process in genes associated with inherited retinal degeneration and Bardet-Biedl syndrome (BBS:MIM#209900). Firstly, we determined if splice site (SS) strength could predict alternative splicing in retinal transcripts. SS strength was calculated on 1400 exons in 60 retinal dystrophy genes using four different scoring methods: Consensus Sequence Weighted Matrices, Neural Network, Information Theory and Maximum Entropy. Based on splice site scoring and EST evidence, exons were classified into low and high SS strength with or without evidence of alternative splicing. RT-PCR on human retinal RNA was performed to validate computational predictions in selected transcripts. High SS strength exons were found to rarely undergo alternative splicing. Exons with low strength splice sites and no EST or RT-PCR evidence of alternative splicing suggest the utilisation of cis-acting elements to regulate splicing. Mutation within these exons are more likely to have a pathological impact on splicing. Secondly, mutant BBS transcripts were classified as vulnerable to aberrant or pathological splicing if sequence variations alter SS strength or splice site definition via splicing enhancers and silencers. SS strength was calculated on 190 exons in 12 BBS genes and potential mutational effects on exonic splicing enhancers and silencers were modelled using the following servers: ESEfinder, FAS-ESS, PESXs, and Rescue-ESE. 290 mutations were modelled and 21% identified in-silico as potential mis-splicing mutations. BBS9, BBS10 and BBS5 genes contained the greatest percentage of possible splicing mutations with 75%, 33% and 33% of mutations modeled affecting either SS strength or splicing enhancers respectively. A number of predicted aberrant splicing events were modelled with a minigene system in HEK293 cell lines to validate predictions. Here, we present an approach to predict and model aberrant splicing in mutant genes.

Common variants in genes underlying mendelian disorders of hypotension and hypertension: association with serum calcium and blood pressure in the general population. *M. D. Tobin[1], M. Tomaszewski[2], P. S. Braund[2], C. Hajat[1], S. M. Raleigh[2], T. M. Palmer[1], M. Caulfield[3], P. R. Burton[1], N. J. Samani[2]* 1) Genetic Epidemiology Group, Departments of Health Sciences and Genetics, University of Leicester, UK; 2) Department of Cardiovascular Sciences, University of Leicester, Leicester, UK; 3) William Harvey Research Institute, London, UK.

In 2037 white European subjects from 520 nuclear families recruited from Leicestershire, England (Genetic Regulation of Arterial Pressure of Humans in the Community - GRAPHIC study), we studied the effects on 24-hour ambulatory blood pressure (BP)and serum cations of common sequence variation in genes underlying mendelian syndromes of hypertension and hypotension. We genotyped 298 common (minor allele frequency>10%) tagging and putative functional SNPs in 11 such regions. Association tests were undertaken using generalized estimating equations (GEEs) and generalised linear mixed models implemented using Gibbs sampling in WinBUGS to account for familial correlations. We adjusted for age, age^2 and sex as covariates and for antihypertensive treatment using a semi-parametric algorithm. Associations were tested with 224 SNPs (75%) passing quality control. In KCNJ1, the type II Bartter syndrome gene which encodes the potassium channel ROMK, we found that five SNPs were associated with 24hour systolic and/or diastolic BP with false positive report probability (FPRP) <0.2 suggesting genuine association. The minor allele of rs2846679 was associated with -1.58 (95% CI -2.47 to -0.69) mmHg change in SBP (P=0.00048, FPRP=0.05). Furthermore, non-synonymous SNP rs1801725 in the calcium-sensing receptor (CASR) SNP, was strongly associated with serum calcium - each minor (T) allele copy was associated with >¼SD change in serum calcium i.e. 22.1 (95% CI 13.8 to 30.4) micromoles, P=1.86*10^{-7}. Epidemiological associations have been reported between serum calcium and cardiovascular traits, including BP. Given the substantial estimated effect size in our study, rs1801725 could prove to be a valuable instrument for mendelian randomization studies that use such genetic data to circumvent confounding and reverse causation in epidemiological studies.

Polymorphism of the leptin gene, leptin concentration and lifestyle habits in the obesity. *K.-T. Chen[1], R.-Y. Wang[2], C.-Y. Chen[2], C.-J. Lin[3], W.-C. Shy[3], M.-C. Hung[3], T.-N. Wu[1], F.-Y. Wu[1]* 1) China Medical University, Environmental Health, Taichung, Taiwan; 2) China Medical University, Public Health, Taichung, Taiwan; 3) China Medical University, Nursing, Taichung, Taiwan.

The aim of our study was to investigate the relationship among leptin gene (LEP), serum leptin concentration, lifestyle habits and other comorbidities (such as high blood pressure and diabetes) in the obesity. The residents in Hsin-I town of Nantou County and in the central-western district of Taichung City participating in the National Adult Health Examination Projoect in Year 2007 were recruited as the study subjects. A total of 682 subjects were obtained. Among them, 220 subjects (32%) with BMI27 were classified as obese and the remainder (68%) with BMI27 were non-obese. Our results showed that there were no statistically significant differences in distribution of the leptin gene (G-2548A) between obese and non-obese individuals. Subjects were divided into four groups based on leptin concentration values: low concentration (leptin4.39), medium concentration (4.39leptin8.84), medium to high concentration (8.84leptin17.64) and high concentration (leptin17.64). Using a proportional odds model analysis we found that as leptin concentration increased, the proportion of those with BMI27 and central obesity also increased. Based on the 2-test we found no relationship among single nucleotide polymorphisms (SNPs) in LEP G-2548A, leptin concentration and obesity. We divided the sample into two age groups: 40-59 yrs and 60 yrs for analysis. In those aged 40-59, we found that obesity was associated with cardiovascular disease, hypertension, blood lipid profile, diabetes mellitus, gout, smoking, alcohol consumption and regular exercise. However, in those aged 60 and over, we found that only cardiovascular disease and regular exercise were associated with obesity. This study suggested that a postivie association exist between the serum concentration of leptin and the risk of obesity. No relationships were found among SNPs in LEP G-2548A, obesity and leptin concentration. In older age groups there were less comorbidities and lifestyle habits were healthier.

N-Acetylmannosamine for the Treatment of Muscle and Kidney Disease: From Mouse to Bedside. *I. Manoli[1,2], E. Klootwijk[1], S. Sparks[1], M. Ziats[1], D. Hickey[1], C. Ciccone[1], P. Zerfas[3], M. Starost[3], D. Darvish[4], D. Krasnewich[1], W. Gahl[1], M. Huizing[1]* 1) Section on Human Biochemical Genetics, MGB, NHGRI, NIH; 2) Intramural Program of the Office of Rare Diseases, NIH; 3) Division of Veterinary Resources, NIH, Bethesda, MD; 4) HIBM Research Group, Encino, Ca.

Hereditary Inclusion Body Myopathy (HIBM) is an adult onset, autosomal recessive neuromuscular disorder characterized by progressive muscle weakness resulting in severe incapacitation within 10 to 20 years. The causative gene, GNE, codes for the enzyme UDP N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase (GNE/MNK), which catalyzes the first 2 steps in sialic acid (SA) synthesis. Paucity of SA presumably causes decreased sialylation of muscle glycoproteins, resulting in muscle degeneration. N-Acetyl-mannosamine (ManNAc), an uncharged sugar, can readily enter cells and serve as a precursor for SA synthesis, not subject to feedback inhibition. We previously administered sialic acid to 4 HIBM patients via intravenous immune globulin (which contains 8mol of sialic acid/g), achieving improved muscle strength and function. We also created knock-in mice harboring the M712T Gne/Mnk founder mutation. Homozygous mutant mice did not survive beyond postnatal day 3 (P3) due to glomerular hematuria, proteinuria, and podocytopathy. Administration of ManNAc yielded survival beyond P3 in 43% of the homozygous pups. Survivors exhibited improved renal histology, increased sialylation of podocalyxin, and increased Gne-epimerase activities. Based on these findings, we now describe a phase I/II, randomized, placebo-controlled, two period cross-over study to determine the safety and efficacy of ManNAc therapy in HIBM. The primary outcome parameter will be change in quadriceps muscle strength. This protocol has assumed increased relevance because our mouse model survivors have developed muscular tubular aggregates and vacuoles at 8-11 months. Our ManNAc trial is also pertinent to renal disorders such as minimal change nephrosis. We will discuss the Rapid Access to Interventional Development Program and the difficulty of performing pre-clinical studies for drugs not approved by the FDA.

A novel mutation at stop codon of the GJA1 gene in Korean patient with congenital heart disease. *M. Hong[1], E. J. Seo[1,2], I. S. Park[1,3]* 1) Genome Research Center for Bir, Asan Medical Center, Seoul, Korea; 2) Department of Laboratory Medicine, University of Ulsan College of Medicine and Asan Medical Center, Seoul, Korea; 3) Department of Pediatrics, University of Ulsan College of Medicine and Asan Medical Center, Seoul, Korea.

Congenital heart diseases (CHD) are the most common developmental anomalies and are diagnosed in 1% of newborns. Although the causes of CHD are mostly unknown, genetic components for CHD have been elucicated through microdeletion syndromes or single gene disorders associated with CHD. GJA1 (gap junction protein alpha 1 43kDa, connexin-43), the main protein of the human cardiac gap junctions, has been shown that GJA1 plays an essential role in the regulation of the development of the heart outflow tract. The mutations in the GJA1 (Cx43) are associated with CHD. Mutation analysis was performed for the GJA1 gene in 116 patients with CHD by PCR based sequencing method. We found only one mutation in 12-year old Korean girl with double outlet right ventricle. This was the novel variation at the stop codon of the GJA1 which can result in the production of abnormal connexin-43 with additional 4 amino acids translated from the 3 UTR of the GJA1: c.1147T>C (TAG>CAG), p.X383GlnextX*4 The variation was not found in 246 control chromosomes, but identified in patients mother who was apparently normal. To evaluate the functional defect of GJA1 caused by X383GlnextX*4, X383GlnextX*4 mutant was constructed using the site-directed mutagenesis and then examined the localization of the transient expression in mammalian cells. The localization studies revealed no significant trafficking defect of X383GlnextX*4 mutant, which was found in both the Golgi apparatus and endoplasmic reticulum, not unlike wild-type GJA1. Further, we will confirm whether the mutant was transported to the plasma membrane in both HeLa and NRK cells.

Genome-wide meta-analyses of haematological traits reveal several novel loci associated with full blood counts.

N. Soranzo[1,2], C. Meisinger[3], N. Watkins[4], A. Attwood[4], P. Deloukas[1], A. Döring[3], M. Falchi[2,5], C. Gieger[3], T. Illig[3], J. Jolley[4], S. Menzel[2], W. H. Ouwehand[1,4], H. Prokisch[3,6], A. Rankin[3], D. Rosskopf[7], T. D. Spector[2], J. Stephens[4], S. L. Thein[2], E. Wichmann[3,8] 1) Wellcome Trust Sanger Institute, Hinxton, UK; 2) Twin Research Unit, Kings College London, London, UK; 3) Helmholtz Zentrum, Muenchen, Germany; 4) University of Cambridge & NHS Blood and Transplant (NHSBT), Cambridge, UK; 5) Imperial College London, London, UK; 6) Technical University Munich, Munich, Germany; 7) Ernst-Moritz-Arndt University, Greifswald, Germany; 8) Ludwig-Maximilians-Universität, Munich, Germany.

Quantitative variation in haematological traits has been linked to cardiovascular disease risk. A recent collaborative effort between German and UK groups aims to discover genetic loci affecting blood cell parameters and cardiovascular disease risk. We have undertaken genome-wide meta-analyses of eight full blood traits at 2.5 million SNPs in 5,000 individuals from three population-based cohorts (TwinsUK, the UK Blood Services Common Control Collection and KORA) and replication in 5,000 samples. A first analysis has identified several novel loci with strong and reproducible evidence for association. Three novel loci were associated with mean platelet volume. These include SNPs in or near *WDR66* (n=10,087, P-value =5.18×10^{-47}), *ARHGEF3* (n= 10,215, P-value =2.45×10^{-25}) and *PIK3CG/FLJ36031* (n=7,417, P-value =9.5×10^{-21}). In addition, we describe one novel locus associated with platelet count on 12q24.12 near *BRAP* (n=6,928, P-value = 2.42×10^{-10}) and replicate associations of the *MYB-HBS1L* locus with increased mean red blood cell haemoglobin content (n=7,268, P-value = 5.4×10^{-27}), increased mean red blood cell volume n=7,270, P-value =8.0×10^{-28}) and decreased red blood cell count (n=7,271, P-value =4.6×10^{-20}). Transcripts of all genes are present in all haematopoietic lineages albeit a different levels. Currently ongoing studies include determination of the effect of the SNPs on the transcript levels in the megakaryocytic, lymphoid and monocytic lineages and on the platelet functional response to activation with ADP and collagen.

Marinesco Sjögren syndrome (MSS): Novel SIL1 mutations and description of a less severe phenotype. *A. Roos[1], M. Baudis[2], R. S. Finkel[3], F. Stanzial[4], C. Stendel[7], A. Dufke[5], H. Topaloglu[6], K. Zerres[1], J. Senderek[1,7]* 1) Institute of Human Genetics, Aachen University of Technology, Aachen, Germany; 2) Institute of Molecular Biology, University of Zurich, Zurich, Switzerland; 3) Division of Neurology, The Children's Hospital of Philadelphia, Philadelphia, USA; 4) Institute of Genetic Medicine, European Academy, Bolzano, Italy; 5) Institute of Human Genetics, Eberhard Karls University, Tübingen, Germany; 6) Child Neurology Unit, Department of Pediatrics, Hacettepe University, Ankara, Turkey; 7) Institute of Cell Biology, ETH Zurich, Zurich, Switzerland.

Marinesco Sjögren syndrome (MSS) is a progressive multisystem disorder with autosomal recessive inheritance. The phenotype is characterized by cerebellar ataxia, congenital or infantile cataracts, progressive vacuolar myopathy, mental retardation, and short stature. Recently, mutations in the SIL1 gene, which encodes an endoplasmic reticulum (ER) resident cochaperone, were identified as a major cause of MSS. Here we describe the results of SIL1 mutation analysis in an extended cohort of patients with MSS or MSS-like conditions. We report five novel mutations in the SIL1 gene, including the first multi-exon deletion described in MSS. We could corroborate our earlier observation that cataracts in MSS are not always present at birth, and we also identified two unrelated patients with SIL1 mutations without mental retardation. In agreement with earlier observations, a subgroup of MSS patients did not carry SIL1 mutations. Our data extend the mutational and phenotypic spectrum associated with the SIL1 gene and confirm genetic heterogeneity in MSS.

Comparison of different methods to control for stratification in genome-wide association studies. *E. Salvi[1,2], G. Guffanti[1], A. Orro[2], S. Lupoli[3], F. Torri[1], S. Potkin[4], C. Barlassina[1], D. Cusi[1], L. Milanesi[2], F. Macciardi[1]* 1) Department of Science and Biomedical Technologies, University of Milan, Milan, Italy; 2) Institute of Biomedical Technologies CNR, Segrate (Mi), Italy; 3) INSPE, Milan, Italy; 4) Department of Psychiatry and Human Behavior University of California, Irvine (CA), USA.

In case-control association studies, population stratification (PS) occurs when allele frequencies differ between cases and controls due to ethnic background or even to hidden; stratification. PS can lead to spurious findings between a phenotype and unlinked candidate loci bringing either to false positive or false negative results when analyzing SNPs for association. To overcome these problems, different strategies have been proposed but currently there is no consensus about a common powerful strategy to adjust for PS. In the presented study, we evaluated different approaches in two different case-control studies of schizophrenia, in order to find the most efficient strategy to achieve enough power to correct the association results and to find the genetic variants really relevant for the disease by avoiding false-positive associations. We assessed PS in a case-control sample of 200 American subjects genotyped for 317K SNPs (Illumina HumanHap300) and in the sample containing 741 schizophrenics of the CATIE project matched with 751 controls collected from the NIMH Genetics repository of which genotype (Perlegen, Affymetrix 500K) and phenotype data are available to the scientific community. Both samples are composed by different ethnic groups. In particular, after identifying the presence of sub-structure, we compared the resulting datasets of the most significant associated SNPs before and after correction, obtained with EIGENSTRAT, PLINK and Genomic Control and we identified the SNPs associated with diseases, the false-positive and the false-negative associations. Indeed a key point is that the PS analysis corrects for false-positive associations but also helps in rescuing potential false-negatives. Each method have advantages and disadvantages and then we decided to use more than one and to follow a workflow of analysis, here presented.

A Homozygous Mutation in *ADAMTSL4* Causes Isolated Ectopia Lentis. *D. Ahram[1], H. El-Shanti[1,2], TS. Sato[2], S. Chen[2], MK. Tayeh[2], A. Kohailan[1], S. Leal[3], M. Al-Salem[4]* 1) SMGC, Doha, Qatar; 2) Univ Iowa, Iowa City, Iowa; 3) Baylor College of Medicine, Houston, TX; 4) JUST, Irbid, Jordan.

Background: Ectopia lentis is characterized by the subluxation of the lens due to the disruption of the zonular fibers, often in association with systemic disorders such as Marfan syndrome. Isolated simple ectopia lentis is reported in families with autosomal inheritance, with dominant forms being more common than recessive. We here report on the mapping and identification of the gene responsible for autosomal recessive isolated ectopia lentis in an Arab family.

Patients, materials and methods: The inbred family has 15 affecteds from 4 related sibships. Clinical and biological data were available from 4 affecteds, 3 obligate carriers and 3 normal siblings. To map and identify the gene, we performed homozygosity mapping using a whole genome linkage mapping set. LOD score analysis was performed using MLINK of the LINKAGE 5.1 computer package. Mutation screening of candidate genes was performed using direct sequencing.

Results: The gene responsible for ectopia lentis in this family was mapped to the pericentromeric region of chromosome 1 (1p13.2 1q21.1), between D1S1675 and D1S498, with a maximum pair-wise LOD score of 3.26 at D1S534 and a =0. We examined 4 candidate genes before the identification of a nonsense mutation in exon 11 of *ADAMTSL4* (Y595X; c.1785TG). This mutation segregates with the disease in the family and was not found in 200 ethnically-matched controls. *ADAMTSL4* is a disintegrin and metalloproteinase with 7 thrombospondin motifs. It is ubiquitously expressed and thought to play a role in the regulation of apoptosis. The mutation produces a truncated protein of half the original length and without 6 of the 7 thrombospondin motifs, thus, it is likely to be nonfunctional.

Conclusion: We conclude that mutations in *ADAMTSL4* are responsible for autosomal recessive simple ectopia lentis. It is speculated that *ADAMTSL4* plays a role in the development and/or integrity of the zonular fibers.

Mathematical characterization of complex disease: Analytic expressions for the multi-locus additive model. *F. Takeuchi, R. McGinnis* Wellcome Trust Sanger Institute, Hinxton, Cambridge, United Kingdom.

The degree of enrichment of disease-causing alleles in affecteds versus unaffecteds is perhaps the most important parameter determining detectability of loci responsible for complex diseases. In single-locus algebraic models of disease, the enrichment is markedly increased in related affecteds (RAs) from multiplex families compared to unrelated affecteds (UAs) from simplex families. However, the enrichment can be influenced by other disease-causing loci ("background loci") distinct from the locus being tested for association with disease (the "foreground locus"). Yet despite the important influence of background loci in modulating enrichment of disease alleles in RAs, this influence has not been studied extensively for one of the two main multi-locus algebraic models (i.e. the "additive"; model in which risks conferred by each locus are added to determine multi-locus penetrance). Furthermore, the few published investigations have relied mainly on simulations. We report here success in deriving analytic expressions that describe the multi-locus additive model including enrichment of disease allele frequency in RAs. One of our key findings is that the frequency of a disease-predisposing foreground genotype in RAs varies between the highly enriched frequency in the single-locus model and the unenriched frequency in unaffecteds. The degree of enrichment within this range is determined by the fraction of the disease recurrence risk among relateds ($_R$) which is contributed by the foreground locus as a proportion of total $_R$ for the entire disease (i.e. foreground plus background loci). We have compared results from our derived equations with published simulation results for specific multi-locus additive models examined by Howson et al. (Genet Epidemiol 29:51, 2005) and Li et al. (Am J Hum Genet 78:778, 2006), and find close agreement with their results. We will illustrate how our analytic expressions can be used to address important issues such as appropriate study design and detectability of foreground disease loci by association testing given known magnitudes of familial aggregation ($_R$) in the particular disease being studied.

Methylation of the Androgen Receptor gene differs between dermal papilla and outer root sheath of human hair follicles. *J. E. Cobb[1], L. Yip[1, 2], N. Wong[3], S. B. Harrap[1], J. A. Ellis[1, 3]* 1) Physiology, University of Melbourne, Parkville, Victoria, Australia; 2) Dermatology, St Vincent's Hospital, Victoria, Australia; 3) Murdoch Childrens Research Institute, Victoria, Australia.

DNA methylation is known to regulate the expression levels of genes in specific tissues via CpG islands within the promoter region. Hair follicles provide us with an easily accessible source of tissue to investigate methylation patterns in humans. The genetic association of the Androgen Receptor gene (*AR*) and Male Pattern Baldness (MPB) has been well documented, and it would appear that *AR* confers strong risk of developing hair loss. Increased *AR* gene expression has been identified in balding (vertex) compared to non-balding (occipital) regions in the same individual, and the lower occipital expression of *AR* may contribute to retention of hair in this region. We therefore hypothesised that in hair follicles from occipital regions of MPB scalp the *AR* promoter is methylated, and thus less expressed compared to vertex hairs. With this in mind we were interested to investigate methylation at the *AR* promoter region initially in two tissues within occipital hair follicles- the dermal papilla (DP) and the outer root sheath (ORS). It is known that *AR* is expressed in both DP and ORS, however while DP is fundamental to maintaining hair growth, ORS can be more easily obtained via plucked hair rather than scalp biopsy. We collected occipital follicle biopsies from hair transplant surgeons and dissected both DP and ORS. The DNA was bisulfite converted, then base-specific cleavage and mass spectrometry was used to determine the pattern of *AR* promoter methylation . Generally, in occipital follicles, we observe DP to have increased methylation at the *AR* promoter compared with ORS. The different methylation patterns within such small and closely located tissues highlights the importance of investigating the specific tissue of interest for human methylation studies. Further studies comparing DP between balding and occipital follicles are planned to determine if increased methylation of *AR* might provide protection to the occipital scalp from androgen-driven hair loss.

Fish Odor Syndrome: Identification of Novel Moroccan Jewish mutation. *T. Yardeni, K. Geva-dayan, N. Goldstein, Y. Anikster* Metabolic Dept , Safra childre'n's hospital Sheba medical center, Tel Hashomer , Israel.

Background: Trimethylamine (TMA) is a malodorous metabolite synthesized by enteric bacteria from dietary precursors. It is normally oxidized to the odorless metabolite trimethylamine N-oxidase by the hepatic enzyme flavin containing monooxygenase 3 (FMO3). Mutations in the FMO3 gene cause the rare autosomal recessive disorder called Trimethylaminuria, also known as the Fish Odor Syndrome. Affected individuals excrete excessive amounts of TMA in body secretions. They suffer from a repelling smell which is exacerbated by certain triggers such as specific foods, especially sea fish, stress, menstruation, puberty and physical exertion. These can lead to disturbance in body perception and to psychosocial problems. It is initially recognizable at a variable age from childhood to adulthood. Methods and Results: We evaluated a family of six siblings, among whom three presented with a long medical history of fishy body odor. Both their parents were of Moroccan Jewish descent, and were first cousins, once removed. Results of molecular genetic studies revealed that they were homozygous for a novel nonsense mutation K4X (c.10A T) in exon 1. Definitive diagnosis enables genetic counseling and treatment strategy planning such as nutritional support, vitamin supplementation and drug therapy. Conclusions: Physicians should be aware of the fact that disturbing body odor may result from a biochemical defect. With the aid of molecular tools for diagnosis this family can find relief in that their unusual complaint is medical and not hygiene related. Genetic counseling and treatment options should be discussed with families diagnosed with trimethylaminuria.

Lack of Association of Caucasian Rheumatoid Arthritis Susceptibility Loci in a Korean Population. *HS. Lee[1, 2], B. D. Korman[3], J. M. Le[3], D. L. Kastner[3], E. F. Remmers[3], P. K. Gregersen[2], SC. Bae[1]* 1) Hanyang University College of Medicine and the Hospital for Rheumatic Diseases, Seoul, South Korea; 2) The Feinstein Institute for Medical Research, North Shore Long Island Jewish Health System, Manhasset, New York; 3) The National Institute of Arthritis and Musculoskeletal and Skin Diseases, Bethesda, Maryland.

Objective: Recent advances in genetics and technology have led to the identification of a number of novel rheumatoid arthritis loci. In Asian populations, PADI4, FCRL3, SLC22A4, and STAT4 have been shown to be associated with disease while significant associations have been identified at PTPN22, TRAF1/C5, 6q23, STAT4, 4q27, CD40, and CCL21 in Caucasian populations. In this study, we sought to determine whether some of the loci identified in Caucasians are also associated with RA in a Korean case-control collection. Methods: We designed a Sequenom iPlex experiment to do a thorough investigation of the PTPN22 linkage disequilibrium region using tag SNPs and to genotype single disease-associated SNPs at other 5 other previously reported Caucasian RA-associated loci in 1123 RA Korean RA patients and 1008 ethnically matched controls. We also re-sequenced the PTPN22 gene to look for novel coding variants that might be contributing to disease in this population. Results: None of the Caucasian RA susceptibility loci contributed significantly to disease in Koreans. Tag SNPs covering the PTPN22 linkage disequilibrium block, while polymorphic, did not reveal any disease association and re-sequencing did not identify any new common coding variants in this population. The 6q23 and 4q27 SNPs assayed were non-polymorphic in this population while the TRAF1/C5, CD40, and CCL21 SNPs did not show any evidence for association. Conclusions: Caucasian and Korean rheumatoid arthritis have different genetic risk factors. While patients of different ethnic groups share the HLA region as a major genetic risk locus, most other genes shown to be significantly associated with disease in Caucasians appear not to play a role in Korean RA.

Identification of Loci for Systemic Lupus Erythematosus by Pooling-based Genomewide Association Study. *T. Tahira[1], K. Masumoto[1], Y. Kukita[1], Y. Okazaki[1], A. Yoshinaga[1], K. Higasa[1], T. Horiuchi[2], K. Hayashi[1]* 1) Div Genome Analysis, Med Inst Bioreg, Kyushu Univ, Fukuoka, Japan; 2) Med. Biosys. Science, Grad. School Med. Sciences, Kyushu Univ, Fukuoka, Japan.

Systemic lupus erythematosus (SLE) is an autoimmune disease in which multiple genetic and environmental factors are involved. Recent genomewide studies of European samples identified genes associated with the disease. However, at least some of them are unlikely to explain the disease in Asians because of the absence of the candidate alleles among the population. To identify the genetic background of SLE susceptibility in Japanese, we performed two-stage pooling-based association study, that is, by array-based hybridization analysis and by SSCP analysis, of case pools (sizes 264 and 183) and control pools (sizes 426 and 253). Each of the pooled samples was hybridized to six Affymetrix 500K array sets and the relative allele signal score (RAS) of each SNP was calculated from intensity signals of perfect-match probes. The disease association was evaluated both by z-score calculated from difference of averaged RAS values between cases and controls and by silhouette score (calculated by Genepool 0.8.1, http://genepool.tgen.org/) and interpreted by a sliding-window statistics of median rank in different window sizes (3, 5, 7, 9, 11, 15 bps). SNPs of highest scores in the top 5 regions for each window size were re-examined by quantitative SSCP (http://qsnp.gen.kyushu-u.ac.jp/placeSSCP/), and the associations (estimated $P < 10^{-4}$) for 22 SNPs in 13 regions were confirmed. The associations of these SNPs were further verified by individual genotyping using TaqMan assay. Most significant associations were found in ZPBP-IKZF1 (upstream of IKZF1, rs876039) and TNFAIP3 (a perfect proxy for non-synonymous SNP, rs2230926). Genotyping of additional samples replicated these associations, and allelic odds ratios were 1.57 ($P = 3.0 \times 10^{-9}$) for rs876039 and 2.1 ($P = 1.1 \times 10^{-8}$) for rs2230926 in the combined sample (630 cases and 988 controls). Associations ($P < 10^{-5}$) were also found near the previously reported regions, i. e. C8orf13-BLK, PRDM1-ATG5 and HLA-G.

Genetic predisposition to femoral neck stress fractures via reduced weight and body mass in military conscripts.

M. Mannikko[1], J. Korvala[1], H. Hartikka[1], H. Pihlajamaki[2,3], S. Solovieva[4], J.-P. Ruohola[3], T. Sahi[3,5], S. Barral[6], J. Ott[6], L. Ala-Kokko[1,7] 1) Department of Medical Biochemistry and Molecular Biology, University of Oulu, Finland; 2) Department of Surgery, National Military Hospital, Helsinki, Finland; 3) Institute of Military Medicine, National Military Hospital, Helsinki, Finland; 4) Finnish Institute of Occupational Health, Helsinki, Finland; 5) Department of Public Health, University of Helsinki, Helsinki, Finland; 6) Laboratory of Statistical Genetics, Rockefeller University, New York, USA; 7) Connective Tissue Gene Tests, Allentown, USA.

Stress fractures are a significant problem among athletes and soldiers. Femoral neck stress fracture is the most severe type, because it can result in devastating problems or even permanent handicap. Genetic factors may increase the fracture risk, but no susceptibility genes have yet been identified. Eight genes involved in bone metabolism or pathology were studied in terms of their roles in femoral neck stress fractures. A case-control association study of 72 military conscripts with a femoral neck stress fracture and 120 controls was performed. Search for predisposing sequence variations in the coding regions of the COL1A1, COL1A2, OPG, ESR1 and LRP5 genes was carried out, and 15 SNPs from the COL1A1, COL1A2, VDR, CTR, LRP5 and IL-6 genes were genotyped. Haplotype analyses were performed on the COL1A1, COL1A2, VDR and LRP5 genes. The LRP5 haplotype A-G-G-C associated to low weight and body mass index (BMI) leading to 2.72 times (95 % CI 1.10-6.73) higher risk of stress fractures in cases than in control subjects. The LRP5 haplotype seems to affect the development of stress fractures in an indirect manner, via reduced BMI. A significant interaction between LRP5 haplotype A-G-G-C and VDR haplotype C-A was also observed and with combined haplotype with 3.85 times higher risk of having femoral neck fractures in cases than in controls (95 % CI 1.16-12.84). These results indicate the first genetic risk factors for development of stress fractures: LRP5 haplotype A-G-G-C increasing the risk of stress fractures via reduced weight and BMI, and an additive effect when in combination with the VDR C-A haplotype.

Neuronal expression of genes involved in synaptic regulation is altered during aging in a mouse model for Parkinson's disease. *B. Chase[1], K. Markopoulou[2]* 1) Dept Biol, AH514B, Univ Nebraska, Omaha, NE; 2) Dept. Neurol, Univ Thessaly Med Sch, Larissa Greece.

-Synuclein overexpression has provided useful animal models for Parkinson's disease (PD). To comprehensively address the spectrum of CNS pathways affected by -synuclein overexpression and how these are altered during aging, we compared neuronal gene expression in pathologically characterized transgenic C57BL6 mice where human -synuclein was expressed under the PDGF- promoter to that in littermate, non-transgenic controls at ages 2 and 11 months. Gene expression profiles were obtained using Affymetrix 430 2.0 arrays (n=5 per group), analyzed using GCOS, Affyminer and Ingenuity software, and confirmed using qRT-PCR. We identified replicable robust changes in gene expression patterns that reflected significant co-regulation of genes lying in networks of interacting proteins affecting the G-protein coupled receptor, axonal guidance, cAMP, LTP, LDP, dopamine, and calcium signaling pathways. While genes in some pathways are affected in the frontal cortex and midbrain at 2 months of age, the number of signaling-pathway genes with altered expression dramatically increases in those areas in 11-month-old mice. Two features are particularly striking. First, genes with altered expression often share similar, key signal transduction functions, e.g., genes for distinct calmodulins, CAMII kinases, and adenylate cyclases are differentially expressed during aging in different pathways. Second, some genes with altered neuronal expression (CDC42, NTNG1, PLXNA2, SLITRK6, UNC18) are identical or function similarly to a subset of the axon-guidance-pathway genes whose SNPs predict PD outcomes in a whole-genome-association study (Lesnick et al., 2007). We conclude that -synuclein overexpression during aging significantly affects genes involved in synaptic regulation and transmission, and that similar key functions are often affected in different signaling pathways. Disregulation in synaptic activity may play an important role in the progression of the neurodegenerative process and perturbations to shared functions in signal transduction may underlie this disregulation.

Genome wide association analysis identifies additional loci affecting adult height in the isolated population of Sorbs in Germany. *A. Tonjes[1], P. Kovacs[1], M. Koriath[1], Y. Bottcher[1], D. Schleinitz[1], K. Dietrich[1], B. Enigk[1], K. Krohn[1], E. Zeggini[2], W. Rayner[2], M. I. McCarthy[2], M. Stumvoll[1]* 1) University of Leipzig, Leipzig, Germany; 2) WTCHG, University of Oxford, Oxford, UK.

Adult height is a strongly inherited trait. Association of a number of common variants with height has recently been reported. We performed a genome wide association study using 500K and 1000K Affymetrix GeneArrays in a recently recruited and extensively phenotyped isolated population from Eastern Germany, the Sorbs. They are of Slavonic origin, and lived in ethnic isolation among the Germanic majority during the past 1100 years. We used linear regression analyses to calculate the effects of genetic polymorphisms on adult height in 929 subjects (59% females, age 47 +/-16.6 years, height 169 +/-9.5 cm). Analyses were corrected for age and gender, for relatedness by using the inflation factor lambda determined by genomic control. Although only part of the recently published SNPs is directly available on the Affymetrix gene array and passed QC in our sample set, we were able to replicate the association of several published variants with adult height in the Sorbs population by use of our genome wide scan (rs4743034 in ZNF462, rs6440003 in ZBTB38, rs6830062 and rs16896068 in LCORL, rs6060373 and rs6088792 in GDF5/UQC, rs2814993 in C6orf106, rs10512248 in PTCH1, rs4794665 near NOG/DGKE/TRIM25/COIL). Furthermore, we identified several new loci associated with height in the Sorbs. Our five strongest associations map in MYBPC1 (p=2.6e-07 for rs11110932), CTNNA2 (p= 4.4e-07 for rs17018086), TMEM108 (p=6.1e-07 for rs9817813), KALRN(p= 7.0e-07 for rs3755702) and in a region in 13q31.1 (p= 3.2e-07 for rs9545880). In conclusion we were able to replicate the effects of previously reported variants in/near ZNF462, ZBTB38, LCORL, C6orf106, GDF5/UQCC, NOG/DGKE and PTCH1 on adult height in the isolated population of Sorbs. Furthermore, we identified additional loci in/near MYBPC1, TMEM108, CTNNA2, KALRN and 13q31.1. We are in the process of extending these findings to a second cohort of German adults as well as children.

Genotyping of the *PORCN* gene in a family with father to daughter transmission of Focal Dermal Hypoplasia (FDH, Goltz syndrome). *D. P. GERMAIN[1], N. MIRI[1, 2], C. BOUCLY[2], P. DE MAZANCOURT[3], M. F. PORTNOI[4]* 1) Department of Genetics, University of Versailles St Quentin en Yvelines (UVSQ). Hopital Raymond Poincare, GARCHES, France; 2) Laboratory of Biochemistry. Hopital Raymond Poincare, GARCHES, France; 3) Laboratory of Biochemistry and Molecular Biology, CHI, POISSY ST GERMAIN, France; 4) Department of Genetics, Hopital Armand Trousseau, PARIS, France.

Background : Focal dermal hypoplasia (FDH) also known as Goltz syndrome (OMIM 305600) is an X-linked dominant disorder of ecto-mesodermal development characterized by patchy dermal hypoplasia with digital, ocular and dental abnormalities. Recently, mutations in the *PORCN* gene which encodes the human homolog of Drosophila melanogaster porcupine, an endoplasmic reticulum protein involved in secretion of Wnt proteins, were demonstrated to cause FDH. The longest splice variant (isoform D) of the *PORCN* gene comprises 15 exons encoding a 1386 bp cDNA that is translated into a protein of 461 amino acids. **Objectives** : To investigate the molecular basis of FDH in a French family where both a 23-year-old girl and her 51-year-old father were clinically affected. **Results** : Mutation analysis by polymerase chain reaction and sequencing of genomic DNA for the entire coding region of the *PORCN* gene in the affected daughter disclosed a nonsense mutation in exon 3, which was not detected in 100 ethnic-matched unrelated control chromosomes. Allele specific restriction analysis of the appropriate PCR fragment confirmed the mutation in the daughter and detected two alleles in the fathers DNA, the mutated allele appearing much weaker than the wild type allele in the male patient. **Conclusion** : Identification of a novel nonsense mutation in the *PORCN* gene confirmed the diagnosis of FDH in a female patient and indicated that she has inherited the disease from her clinically affected father who carries the mutation as a mosaic. Our results also highlights the clinical importance of PORCN and Wnt signalling pathways in embryogenesis.

Crosstalk between NF-B and Wnt/-catenin pathways and anhidrotic ectodermal dysplasia. *C. Cluzeau[1], E. Bal[1], P. Guigue[1], N. Chassaing[2], S. Hadj-Rabia[1,3], C. Bodemer[1,3], P. Calvas[2], M. C. Vincent[2], A. Munnich[1], A. Smahi[1]* 1) INSERM U781, Hôpital Necker-Enfants Malades, Université Paris Descartes, Paris, France; 2) Service de Génétique médicale, Hôpital Purpan, Toulouse, France; 3) Service de Dermatologie, Hôpital Necker-Enfants Malades, Paris, France.

Anhidrotic ectodermal dysplasia (EDA) is an ectodermal differentiation disorder characterized by sparse hair, abnormal or missing teeth and inability to sweat. X-linked EDA, caused by mutations in *ED1* gene encoding ectodysplasin, a member of the TNF family, is the most frequent form. The autosomal dominant and recessive EDA forms, clinically identical to X-linked forms, may result from mutations in two loci : *ED3*, encoding Edar, ectodysplasin receptor and *ED2*, encoding Edaradd, Edar adapter molecule necessary in signal transduction. Edar is activated by ectodysplasin and uses Edaradd as an adapter to activate NF-B signaling pathway. Wnt/-catenin pathway plays a central role during embryonic development and is widely involved in carcinogenesis. This pathway has been recently involved in skin appendages formation. A crosstalk between NF-B and Wnt/-catenin pathway was described. We confirmed by transactivation experiments that Edar receptor inhibits Wnt/-catenin pathway. We then studied the effects of seven recessive and dominant mutations identified in Edar gene on both pathways. Dominant mutations totally impaired NF-B activation and Wnt/-catenin downregulation, while recessives ones only disrupted the Edar effect on the two pathways. We first demonstrated that Wnt/-catenin inhibition by Edar is dependent of NF-B activation using a dominant negative form of IB inhibitor. We then proved by Western Blot analysis and immunofluorescence that -catenin was neither degraded nor delocalized after NF-B activation by Edar. Furthermore, -catenin/TCF4 interaction, necessary for Wnt/-catenin transcriptional activity, was not disrupted upon Edar transfection and NF-B subunits p65 and p50 did not interact with -catenin in these conditions.

Two novel mutations in surfactant protein C, lung function and obstructive lung disease in the general population. *M. Baekvad-Hansen, B. G. Nordestgaard, A. Tybjærg-Hansen, M. Dahl* Copenhagen University Hospital, Faculty of Health Sciences, University of Copenhagen, Denmark.

Background: Mutations in surfactant protein-C (SP-C) have been associated with alveolar proteinosis and pneumonitis in children, and with interstitial lung disease among adults. We hypothesized that genetic variants in SP-C affect pulmonary function and risk of developing obstructive lung disease in adults. Methods: We resequenced SP-C in order to identify new genetic variation. We selected 760 individuals with extreme pulmonary phenotypes from the Copenhagen City Heart Study, a general population study of 10,604 adults. An extreme pulmonary phenotype was defined in those with the highest/lowest lung function, early-onset asthma, early-onset chronic obstructive pulmonary disease or interstitial lung disease. We screened the protein coding region of SP-C in all selected individuals. We subsequently genotyped two large population studies, the Copenhagen City Heart Study (n=10,604) and the Copenhagen General Population Study (n=37,337), in order to assess the clinical relevance of the SP-C mutations detected by extreme phenotype screening. Results: We identified eighteen genetic variants in SP-C, of which two were new mutations. One mutation was amino acid substituting and the other introduced a stop codon. These mutations were situated in the exon 2 and BRICHOS domain of SP-C, two conserved regions that harbour the majority of disease-associated mutations identified up to now. Screening two large and ethnically homogenous populations for these mutations, we found that they did not affect lung function or consistently change risk of developing obstructive lung disease. Conclusion: We identified two novel mutations in two conserved regions of the SP-C gene and show that adults that are heterozygous for these mutations are relatively free from common diseases of the lung. The data suggests lower penetrance than expected of pulmonary disease in SP-C heterozygotes.

CYP1B1 genotype and Risk of Myocardial Infarction, Chronic Obstructive Pulmonary Disease, Cancer, and Early Death. *D. Kaur-Knudsen, S. E. Bojesen, A. Tybjærg-Hansen, B. G. Nordestgaard* Copenhagen University Hospital, Faculty of Health Sciences, University of Copenhagen, Denmark.

Objective: Cytochrome P450 1B1 enzymes play an important role in metabolism of tobacco-smoke substances such as polycyclic aromatic hydrocarbons (PAHs) and of 17-estradiol. CYP 1B1*3 (4326CG, rs. 1056836) and 1B1*4 (4390AG, rs. 1800440) have altered enzyme activity towards metabolism of PAHs and 17-estradiol. We hypothesized that these two polymorphisms predict risk of myocardial infarction (MI), chronic obstructive pulmonary disease (COPD), cancer, and early death. Method: We genotyped and followed prospectively for 31 years 10,398 adults from the Copenhagen City Heart Study (CCHS). Significant results were re-tested cross-sectionally in the Copenhagen General Population Study (CGPS) with 37,178 participants and in a case-control study, the Copenhagen Ischaemic Heart Disase Study (CIHDS), with 2,379 cases and 33,238 controls. Results: In the CCHS, hazard ratio for MI among never smokers was 1.91 (95% CI 1.16-3.15) for CYP1B1*3 GG (19%) vs. CC (32%); however, this was not significant among smokers. On re-testing these findings in the CGPS and CIHDS, we did not find any association, and we could reject odds ratios above 1.5 in CYP1B1*3 GG vs. CC with 90% power. For COPD, cancer, female cancer and early death we found no association to CYP1B1*3 genotype; however; for tobacco-related cancer we observed with a hazard ratio of 0.80 (0.64-0.99) in CYP1B1*3 GG vs. CC. On re-testing these findings in the CGPS, we did not find any association, and we could reject odds ratios below 0.71 in CYP1B1*3 GG vs. CC with 90% power. For CYP1B1*4 genotype we did not find any association with either endpoint. Conclusion: CYP1B1*3 and CYP1B1*4 genotypes do not predict myocardial infarction, chronic obstructive pulmonary disease, cancer, or early death.

Molecular diagnosis of galactosemia in cholestasis of infancy: GALT activity and mutation detection. *R. Prasad*[1], *R. Singh*[1], *G. Kaur*[2], *BR. Thapa*[1] 1) Biochemistry, PGIMER, Chandigarh, India; 2) Government Medical College and Hospital, Chandigarh, India.

Introduction: Galactosemia is a metabolic disorder due to deficiency of galactose-1-phosphate uridyl transferase (GALT) resulting in complications like hepatocellular damage, sepsis and developmental delay in untreated infants. Till date there are no reports on molecular diagnosis from India. Objective: To determine GALT activity in infants with cholestasis and to detect mutations in GALT gene. Materials and Methods: A cohort of 178 infants (5 days -10.5 months), admitted in Pediatric Gastroenterology ward of PGI over a period of 24 months, with cholestasis were evaluated for galactosemia. Basic investigations were done in all patients, urine for non-glucose reducing substances, TORCH serology, abdominal ultrasound, mebrofenin scan (for biliary excretion) and perop cholangiography were done when indicated. Screening for GALT defeciency was done using Perkin-Elmer neonatal GALT kit. DNA was isolated from patients exhibiting decreased GALT activity. Mutation analysis for most common Q188R, N314D and S135L mutations was performed by RFLP. SSCP analysis was done for detection of unknown mutations Results: Twenty seven patients were found to have reduced GALT activity in age group of 1-7 months, M: F 18:9. Jaundice in 27 (100%), hepatomegaly in 27 (100%), splenomegaly in 17 (62%), coagulopathy in 15 (55%), UTI in 6 (22%), encephalopathy in 3 (11%), and septicemia in 3 (11%), were observed. N314D mutation was found to be most common: homozygote n=1 and heterozygotes n=19. Homozygous and heterozygous Q188R mutations were detected in one patient each. Further, we detected two novel mutations - P185L in exon 6 and S307X in exon 10 by SSCP and direct gene sequencing. None of the patient was found to be positive for S135L. Conclusion: Genotype correlation with GALT activity revealed variable enzyme activity in N314D mutation, while reduced activity in other identified mutations. Screening for GALT activity in infants with cholestasis should be undertaken and genetic analysis should be done for confirmation.

Genetic Modifier(s) of Embryonic Lethality in Type IIA Procollagen Deficient Mice. *Y. Q. Song[1], P.LF. Tang[1], S. Y. Y. Wong[1], A. W. L. Leung[1], P. C. Sham[2], K. S. E. Cheah[1]* 1) Dept Biochemistry, Univ Hong Kong, Hong Kong, Hong Kong, China; 2) Department of Psychiatry, University of Hong Kong, Hong Kong SAR, China.

Col2a1 encodes type II procollagen, the major cartilage matrix protein. During embryogenesis Col2a1 is differentially transcribed to give IIA mRNA containing an exon (exon 2) and IIB mRNA, which lacks this exon. We produced IIA procollagen deficient mice by deleting exon 2 of Col2a1 and observed that mice homozygous for the IIA null mutation (IIA-/-) display complex congenital malformations resulting in prenatal lethality to near normal phenotype, depending on the strain background of the mice. Upon backcrossing (6 generations) to C57BL background, an increasing proportion of the IIA-/- mice die in the prenatal period with heart defects. These observations led us to hypothesize that the phenotypic variability in IIA-/- mice were related to differences in the genetic background. Three congenic mouse lines backcrossed over 7 generations to different genetic backgrounds (129/sv; C57BL; ICR) are therefore established to characterize the inheritance model of the modifier(s). Our segregation analysis shows that there is a significantly underrepresention of IIA-/- offspring from a C57BL IIA+/- intercrosscross ($p=0.0137$, $df=2$), however, the ratio appears to be normal for offspring from 129/sv IIA+/-and ICR IIA+/- intercross. This data would be consistent with an inheritance model with one or a set of recessive modifier gene(s) in the C57BL mice interacting with the IIA-/- genotype and results in prenatal lethality. Having understood the characteristics of the modifier(s), we designed a mapping panel to identify its physical location by first crossing ICR IIA+/- x C57BL IIA+/- to obtain F1. IIA-/- F1 would be selected to generate a IIA-/- F2 population with presumably segregating modifier alleles. 8 families with 15 cases, 50 control and 6 unknown were collected using the mentioned mapping panel. Nonparametric linkage analysis was performed on over 200 SNPs covering the whole mouse genome. One marker on chromosome 7 was shown to have significant linkage with our phenotype of interest. Further investigation is being carried out to identify the candidate modifier.

Association of AKT-1 DNA sequence variants with schizophrenia and neurocognitive phenotypes. *D. B. Wildenauer[1], A. C. Doyle[2], M. Albus[3], S. G. Schwab[1,4]* 1) CCRN Sch Psychiatry, UWA, Claremont, Australia; 2) Western Australian Institute for Medical Research, UWA, Perth, Australia; 3) Mental State Hospital Haar, Germany; 4) School of Medicine and Pharmacology, UWA, Perth, Australia.

AKT1 has been suggested as susceptibility gene for schizophrenia by genetic association and functional studies. We tested nine single nucleotide polymorphisms (SNPs) within the gene for AKT1 in a sample of 365 families of German ancestry for association with schizophrenia and with three neurocognitive tests, i.e. trail making test A and B, and Stroop colour naming test. These tests are measures for psychomotor speed, executive function, and reaction time, and indicate neurocognitive deficits. Neurocognitive test results were available for 230 of the 365 families. Genotyping was conducted using fluorescence based technologies. Statistical analysis was performed using FBAT and PBAT. SNP rs10149779 revealed statistical significant association with schizophrenia (P=.03) for the entire sample of 365 families. When analysis was restricted to a sub-sample (N=79 families) with an ascertainment scheme likely to provide greater genetic loading for the disorder, four SNPs were significantly associated with schizophrenia with the smallest P-value revealed by a SNP located in the 5-upstream region of the AKT-1 gene (rs4983559, p=0.0005). Analysis of the neurocognitive phenotypes identified significant associations between three SNPs (rs4983559, rs1130214, and rs10149779) for both versions of the Trail-making test (parts A and B), but not for the Stroop test. Our study replicates and expands previous reports suggesting a role for AKT1 in pathogenesis of schizophrenia.

Mutation meltdown of mitochondrial DNA and Neanderthal extinction. *G. Hudson[1], D. C. Samuels[2], P. F. Chinnery[1]* 1) Mitochondrial Research Group, Newcastle University, Newcastle upon Tyne, Tyne and Wear, United Kingdom; 2) Virginia Bioinformatics Institute, Virginia Polytechnic Institute and State University, Blacksburg, USA.

There is emerging evidence that mitochondrial DNA (mtDNA) plays and integral role in the evolution of the human species. Although contentious, recent phylogenetic studies of modern humans implicate genetic variation of mitochondrial DNA (mtDNA) as a major factor underpinning the climatic adaptation of across the globe. Greater sequence diversity in the *MTATP6* gene in arctic populations led to the idea that specific mtDNA polymorphisms cause subtle uncoupling of the respiratory chain, with the subsequent generation of additional heat being adaptive in northern climes. Our knowledge of mtDNA and its affect on adaptability may help us to understand how modern humans have survived their early ancestors. Here, we characterise the mtDNA of one of these extinct hominids. Neanderthals are the closest hominid relatives of modern humans, who up until 30,000 years ago coexisted in Europe and western Asia. Recently, over 1Mb of DNA was successfully extracted and characterised from the Vi-80 Neanderthal fossil. We reanalysed 2,705 base pairs of mtDNA in order to examine the hypothesis that mitochondrial dysfunction contributed to the Neanderthals demise. We identified thirty-two nucleotide differences from the modern human mtDNA reference sequence and by treating the Vi-80 as a diagnostic sample leads us to the conclusion that sequence variants that are highly likely to be artifacts, and a large proportion of the remaining mutations could be due to nuclear pseudogene amplification. We did identify a potentially deleterious variation; however more study may be needed to ascertain the effect of mitochondrial dysfunction on Neanderthal survival.

Use of genome-wide association to identify predictors of drug efficacy in clinical development. *G. Kazeem[1], SA. Bacanu[1], H. Hong[2], M. Nelson[1], A. Bansal[1], S. Sundseth[1], A. Menius[1], K. Nangle[1], F. Goodsaid[2], B. O'Neil[2], SJ. Wang[2], M. Ehm[1]* 1) GlaxoSmithKline; 2) US Food & Drug Administration.

Several recent successes in genome-wide association (GWA) studies have provided some insights into the genetic architecture that underlies many complex human traits. However, using the GWA approach to identify genetic biomarkers that influence drug efficacy, and that could improve clinical development, remains largely untested. In a collaboration between the FDA and GSK, blinded whole-genome datasets, obtained from three phase IIb and III clinical trials, were analysed under a variety of statistical models. Data include a quantitative baseline and response variables and Affymetrix 500K SNP genotypes from 961 subjects collected from three clinical studies. To identify genetic markers that influence compound efficacy, single-point analysis was performed for each marker adjusting for covariates identified in the clinical studies. To reflect the progressive nature in which the data were obtained, analyses were first performed for the first study data, for the first two datasets combined and then for all the three datasets. Single-point tests were also performed for each study separately. Results of genetic association were interpreted using the Bayesian False Discovery Probability (BFDP). Testing for genotype-by-treatment interaction effect in the combined datasets from the three studies gave no significant association signals. However, when the analyses were restricted to the treatment arm only (testing for genotype main effect among treated subjects), loci with noteworthy effects were observed in each separate study, some of which also came up in the combined datasets (BFDP0.91 threshold for GWA and p-values$2x10^{-6}$). Simulation studies were also performed to determine the effect sizes that can be reliably detected given the phenotype distribution and the sample size. We observed that testing for genotype main effect in treated arm only gave the highest power among the models considered. These results will help to determine how genetic variants that influence drug efficacy can be identified, incorporated into classifiers and tested during the course of clinical development.

Further delineation of Pitt-Hopkins syndrome: phenotypic and genotypic description of sixteen novel patients. *C. Zweier[1], H. Sticht[2], E. K. Bijlsma[3], J. Clayton-Smith[4], S. E. Boonen[5], A. Fryer[6], M. T. Greally[7], N. den Hollander[3], M. Jongmans[8], S. G. Kant[3], M. D. King[9], S. A. Lynch[7], S. McKee[10], A. T. Midro[11], S. Park[12], V. Ricotti[9], E. Tarantino[13], M. Wessels[14], M. Peippo[15], A. Rauch[1]* 1) Institute of Human Genetics, University Erlangen-Nuremberg, Erlangen, Germany; 2) Bioinformatics, Erlangen, Germany; 3) Department of Clinical Genetics, Leiden, The Netherlands; 4) Academic Department of Medical Genetics and Regional Genetics Service, Manchester, UK; 5) Kennedy Center, Glostrup, Denmark; 6) Department of Clinical Genetics, Liverpool, UK; 7) National Centre for Medical Genetics, Crumlin, Dublin, Ireland; 8) Department of Human Genetics, Nijmegen, The Netherlands; 9) Department of Pediatric Neurology, Temple Street, Dublin, Ireland; 10) Northern Ireland Regional Genetics Centre, Belfast, UK; 11) Department of Clinical Genetics, Bialystok, Poland; 12) Department of Clinical Genetics, Cambridge, UK; 13) Section of Clinical Genetics, Pisa, Italy; 14) Department of Clinical Genetics, Rotterdam, The Netherlands; 15) Department of Medical Genetics, Helsinki, Finland.

Haploinsufficiency of the gene encoding for transcription factor 4 (TCF4) was recently identified as the underlying cause of Pitt-Hopkins syndrome, a so far underdiagnosed mental retardation syndrome characterized by a distinct facial gestalt, breathing anomalies and severe mental retardation. To date 11 patients with PTHS and deletions or mutations in the TCF4 were reported. We now performed TCF4 mutational analysis in 117 patients with Pitt-Hopkins syndrome like features and identified 16 novel mutations. All of these proven patients were severely mentally retarded and showed a distinct facial gestalt, 56% had breathing anomalies, 56% had microcephaly, 38% had seizures, and 44% had MRI anomalies. We therefore further delineate the mutational and clinical spectrum of Pitt-Hopkins syndrome and confirm its important role in the differential diagnosis of severe mental retardation, in particular Angelman- and Rett-syndromes.

Polymorphic variants in Tenascin-C are associated with coronary artery disease. *M. A. Minear[1], D. R. Crosslin[1], B. S. Sutton[1], S. C. Nelson[1], S. G. Watson[1], A. B. Hale[1], J. J. Connelly[1], C. Haynes[1], J. M. Vance[3], D. Seo[3], S. H. Shah[1,2], P. J. Goldschmidt-Clermont[3], W. E. Kraus[2], E. R. Hauser[1,2], S. G. Gregory[1,2]* 1) Center for Human Genetics, Duke University, Durham, NC; 2) Department of Medicine, Duke University, Durham, NC; 3) Miller School of Medicine, University of Miami, Miami, FL.

Tenascin-C (TN-C) is an extracellular matrix protein with several functions related to atherosclerosis pathophysiology, including aortic smooth muscle cell proliferation, migration, apoptosis, and neointimal hyperplasia. Previously, TN-C expression patterns were shown to discriminate between and predict atherosclerotic disease states in human aorta samples. Because TN-C is an attractive atherosclerosis candidate gene, we sought to define whether polymorphisms in TN-C were associated with a clinical manifestation of atherosclerosis, coronary artery disease (CAD). To do this, we genotyped tagging SNPs across the TN-C locus in three independent CAD samples: atherosclerotic human aortas, CATHGEN CAD cases and controls, and GENECARD (GC) early-onset CAD families. Single SNP association analyses using Caucasian subjects and multivariable logistic regression to account for known CAD risk factors identified three SNPs (rs4452883, rs3789875, and rs12347433) in high linkage disequilibrium (r^2>0.95) that were consistently associated across all three datasets. The strongest associations were observed with advanced forms of CAD, defined by the presence of raised lesions in aortas (n=222, p-values (p)=0.001-0.004, odds ratios (OR)=3.4-4.5) or by CATHGEN cases with high CAD indexes (n=230, p=0.001-0.008, OR=1.5-1.7) or left main artery disease (n=244, p=0.005-0.01, OR=1.5-1.6). Although not statistically significant, the entire GC dataset showed trends of association using the APL test (n=2954, p=0.09, rs12347433) and by comparing GC probands to unaffected controls (n=650, p=0.07-0.09, OR=1.3). The association with rs12347433, a synonymous SNP (sSNP), is noteworthy given recent studies showing that sSNPs can alter mRNA stability, translation kinetics, or alternate splicing. This work highlights the importance of TN-C as a candidate gene for CAD.

Large scale population carrier screening for spinal muscular atrophy (SMA) in Israel - effect of ethnicity on the false negative rate. *R. Sukenik-Halevy[1], R. Pesso[2], N. Garbian[2], N. Magal[1], M. Shohat[1,2]* 1) Raphael Recanati Genetic Institute, Rabin Medical Center, Petah Tikva, Israel; 2) Maccabi Health Insurance Genetic Institutes, Rehovot, Israel.

Approximately 95% of Spinal Muscular Atrophy (SMA) patients have homozygous deletions of exon 7 and/or 8 of the SMN1 gene and 5% are compound heterozygotes for deletion of exon 7 and a point mutation. Based on patient prevalence studies, the carrier frequency has been estimated to vary from 1:150 to 1:35. We undertook a survey to assess the carrier rate among healthy individuals with no family history, evaluate the false negative rate (individuals with three copies of exon 7), and determine any ethnic differences. We analyzed data from a total of 9037 subjects who were tested between February and October 2007 in two large medical centers in Israel that conduct carrier screening among the normal population using the MLPA kit. We studied the copy number of exons 7 and 8 and divided the subjects into six ethnic groups: Ashkenazi Jewish, North African Jewish, Iranian and Iraqi Jewish, Yemenite Jewish, Balkan Jewish, and a group of others, which included individuals with a mixed ethnic origin or one that did not fit into one of the six categories. Statistical analysis was performed using the chi-square test. The carrier rate (deletion of exon 7) was 1:62 and was not statistically different among the various ethnic groups. A duplication of exon 7 was found in 1 in 9 (11.1%) individuals - a false negative rate of 5.5%. There was a significant difference between the ethnic groups: 13.3% among Ashkenazim, 6% among North African Jews and 7.7% in Iraqi Jews ($p<0.001$). This difference was also found for duplication of exon 8. The pattern of results was consistent in both testing centers. These findings suggest that the frequency of duplication of exon 7 may in some ethnic groups be several times higher than that of a deleted allele. This emphasizes the importance of determining the false negative rate for each ethnic group as it may vary markedly. The discrepancy between the rates of exon 7 deletions vs. duplications may be explained by the genetic disadvantage of deletions.

Migraine with or without aura is associated with copy number variants in different genes. *L. Armengol[1,2], S. Villatoro[1,2], J. R. González[2,3], K. Rabionet[1], B. Cormand[4], A. Oterino[5], M. Toriello[5], A. Macaya[6], R. Corominas[6], E. Cuenca[6], M. J. Sobrido[7], J. Pardo[8], J. López[8], R. Leira[8], M. Camiña[6], A. Carracedo[7], E. Martí[1,2], X. Estivill[1,2,9]* 1) Genes & Disease Program, Center for Genomic Regulation; 2) CIBERESP; 3) Centre de Recerca en Epidemiologia Ambiental; 4) Departament of Genetics, Universitat of Barcelona; 5) Department of Neurology, Hospital Marqués de Valdecilla, University of Cantabria; 6) Grup Recerca Neurologia Infantil, Hospital Universitari Vall d'Hebron; 7) Fundación Pública Galega de Medicina Xenómica; 8) Department of Neurology, Hospital Clinico Universitario de Santiago; 9) Pompeu Fabra University, Barcelona, Catalonia, Spain.

Migraine is a complex and very prevalent medical condition characterized by recurrent painful headaches. Migraine is known to have a strong genetic component as suggested by the familial aggregation and the high concordance rate observed in monozygotic twins. To assess a possible role of CNVs in migraine etiology, we pooled DNA from migraine patients and hybridized it against a pool of controls on Agilent 244K arrays. Eight loci were found to be concordant among hybridization replicates. We identified the breakpoints of the two most promising CNVs and genotyped copy number variability. Preliminary association studies in a cohort of 451 cases and 448 controls revealed an association between migraine (F-test; p=0.017; OR=1.35; CI [1.06-1.72]) and the presence of the insertion allele of the CNV. The CNV is 8 kb long and lies within a gene that is highly expressed in brain nuclei involved migraine's etiology. Replication studies for the association, linkage with other markers in the region and functional studies are in progress. A second variant identified affects another candidate gene for migraine. Preliminary MLPA analyses on 77 cases and 166 controls reveal that this CNV is also associated with migraine (F-test;p=0.018;OR=1.69;CI [1.09-2.63]). These are, to our knowledge, the first CNVs associated with a complex and highly prevalent neurological disorder. Our work emphasizes the importance of CNVs in the etiology of complex disorders and sheds new light on the biological basis of migraine.

Study of candidate genes for cognitive endophenotypes of schizophrenia on cromosomes 2q33-37 and 4q13-26. *J. Wedenoja[1,2], I. Lindqvist[1], A. Tuulio-Henriksson[3,4], J. Suvisaari[3], A. Loukola[1], T. Varilo[1,2,5], S. Mottaqui-Tabar[1], P. Lahermo[6], E. Serkkola[7], S. Ripatti[1,8], M. Ranki-Pesonen[7], J. Lönnqvist[2,3,9], L. Peltonen[1,2,5,10], T. Paunio[1,3,9]* 1) Institute for Molecular Medicine Finland FIMM, National Public Health Institute, Helsinki, Finland; 2) Department of Medical Genetics, University of Helsinki, Finland; 3) Department of Mental Health and Alcohol Research, National Public Health Institute, Helsinki, Finland; 4) Department of Psychology, University of Helsinki, Helsinki, Finland; 5) Program in Medical and Population Genetics, Broad Institute of Harvard and MIT, Cambridge, MA, USA; 6) Finnish Genome Center, Institute for Molecular Medicine Finland, University of Helsinki, Helsinki, Finland; 7) Research and Development, Orion Pharma, Espoo, Finland; 8) Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden; 9) Department of Psychiatry, Helsinki University Central Hospital, Helsinki, Finland; 10) Human Genetics, Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, UK.

Lack of convincing results in gene identification for psychiatric disorders has increased interest towards quantitative traits which are potentially more closely related to underlying biology. We have previously identified in Finnish schizophrenia families a locus for visual working memory on 2q36 and for verbal learning and memory on 4q21, considered as valid schizophrenia endophenotypes. We selected 70 regional, functionally relevant candidate genes from 2q33-37 and 4q13-26, and genotyped altogether 1144 intragenic tagging SNPs in a nationwide Finnish sample of 293 schizophrenia families with detailed interview and neuropsychological test data. We detected a significant association between visual working memory and PAX3 on 2q36 (p0.00001), and semantic clustering, a trait related to verbal learning and memory, with SNCA on 4q22 (p0.001). Interestingly, these genes are located on the same genomic regions as our best genome-wide linkage loci for the respective traits. Our findings support the hypothesis that the genetic risk related to psychiatric disorder candidate genes is mediated via their effect on cognitive endophenotypes.

mtDNA genetic landscapes formed in tumors and in human evolution are shaped by similar selective constraints.

I. Zhidkov[1,2], E. Livneh[1,2], E. Rubin[1,2], D. Mishmar[1,2] 1) Life Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel; 2) The National Institute of Biotechnology (NIBN), Ben-Gurion University of the Negev, Beer-Sheva, Israel.

Genomic landscapes in humans revealed the signatures of natural selection in mutations generated either during evolution or during the progress of cancer. The unique features of the mutational landscape in cancer are the target of extensive study. However, less attention has been drawn to similarities in the signatures of selection in tumor cells and human populations. We hypothesized, that such similarities will provide new insight into the functional constraints acting on both systems. Here we utilized the rapidly evolving mitochondrial genome (mtDNA) to compare the de novo mutational landscapes in a cancer compendium (98 sequence pairs) with mutations fixed in the mtDNA during human evolution (2400 sequences). Nucleotide positions that underwent de novo changes in the cancer compendium preferentially co-localized with ancient mutations in the human mtDNA phylogeny. An even stronger pattern was observed when recurrent combinations of mutations (COMs) were analyzed in the cancer compendium, revealing non-random COMs of up to seven mutations in length, longer than observed in reshuffling simulations ($p<2.9\times10\text{-}4$). Strikingly, 23/25 positions comprising the COMs co-localized with positions that define major human mtDNA lineages. Intriguingly, the mtDNA COMs included 14 changes not affecting protein sequences or RNA genes (9 synonymous and 5 mtDNA D-loop mutations), suggesting a functional potential for non-coding changes. Our results reveal significant similarities in the mutational landscapes of mtDNA in cancer and normal human populations suggesting similar selective constraints. This implies a functional potential for specific positions as well as combinations of positions in both de novo and inherited mtDNA mutations. Our findings propose new ways to understanding principles in cancer and natural genomic evolution.

A PCSK9 variant in a dyslipidemic disorder other than familial hypercholesterolemia. *M. ABIFADEL[1,2], L. BERNIER[3], G. DUBUC[3], JP. RABES[1,4], J. BONNEAU[1], A. MARQUES[1], M. MARDUEL[1], M. DEVILLERS[1], D. ERLICH[1], A. MUNNICH[1], M. VARRET[1], M. ROY[3], J. DAVIGNON[3], C. BOILEAU[1,4]* 1) INSERM U781, Hopital Necker, Université Paris Descartes, France; 2) Faculté de Pharmacie, Université Saint-Joseph, Beirut, Lebanon; 3) Institut de recherche clinique, Montreal, Canada; 4) Laboratoire de Biochimie et de Génétique Moléculaire, CHU Ambroise Paré (AP-HP et Université Versailles-Saint-Quentin-en-Yvelines), Boulogne, France.

Hypercholesterolemia is one of the major causes of coronary heart disease (CHD). The genes encoding the low density lipoprotein receptor (LDLR) and its ligand apolipoprotein B (APOB), have been the two genes classically implicated in autosomal dominant hypercholesterolemia (ADH). Our discovery in 2003 of the first mutations of PCSK9 gene causing autosomal dominant hypercholesterolemia shed the light on an unknown actor in cholesterol metabolism that has been extensively investigated since. Several PCSK9 variants have been identified, some of them (p.S127R, p.D129G, p.F216L, p.R218S, p.357H, p.D347Y, p.D374H) are gain of function mutations causing ADH or hypercholesterolemia by a reduction of LDL receptor levels; while others are loss of function mutations (p.Y142X, p.C679X) associated with a reduction of LDL-cholesterol levels and a decreased risk of CHD. PCSK9 variants are unequally distributed in different ethnic groups contributing to the variability of cholesterol levels and CHD incidence in these populations. We sequenced PCSK9 in 25 French and French-Canadian probands with ADH with no mutations in the LDLR or APOB genes, and in 25 other French-Canadian dyslipidemic probands. We identified a new variant of PCSK9 in one of the 25 French-Canadian ADH families and in 2 families with dyslipidemia other than ADH. Furthermore this variant was not found in 100 French and 100 French-Canadian controls. Statistical studies and family based test (FBAT) studies showed an association of this variant with the dyslipidemic phenotype present in these families. Thus, in this report we show for the first time that a PCSK9 variant might be implicated in a multifactorial dyslipidemic disorder.

Fine scale analysis of ~1 million genome-wide SNPs on 250 individuals from the HGDP-CEPH panel. *M. Bauchet[1], D. Lopez-Herraez[1], K. Tang[4], I. Pugach[1], M. Scholz[2], A. Gro[2], M. R. Nandineni[3], M. Stoneking[1]* 1) Max Planck Institute for Evolutionary Anthropology, Dept of Evolutionary Genetics, Leipzig, Germany; 2) University of Leipzig, Institut fuer medizinische Informatik, Statistik und Epidemiologie, Leipzig, Germany; 3) Centre for DNA Fingerprinting and Diagnostics, Nacharam, Hyderabad, India; 4) CAS-MPG Partner Institute of Computational Biology, Shanghai, China.

We analyzed genome-wide SNPs typed on a world-wide panel of 250 individuals from the HGDP-CEPH panel, genotyped on Affymetrix Human SNP Array 5.0 chips. Data are available for these same samples from the Illumina HumanHap650K BeadChip; some 96k SNPs overlap between the two arrays. For the Affymetrix arrays, we first devised the best genotyping call method (Chiamo) and optimized its parameters by choosing the set that minimizes mismatches with the Illumina data. We then merged both arrays, filtered out ambiguous and potentially biasing SNPs. We obtained approximately 1 million SNPs for 250 individuals5 from each population of the HGDP-CEPH panel. We performed principal component analysis (PCA) on this dataset at two different thresholds applied to the Chiamo posterior probabilities. We discuss the shape and directions of clustering at the worldwide and regional levels, and identify ancestry informative markers (AIMs) at both geographical levels whenever possible. In addition we phased the merged set and searched for signals of natural selection using contrasting extended haplotype homozygosity (EHH) profiles between populations. These results add substantially to our knowledge of human population history, and provide lists of between and within continent AIMs, as well as candidate genomic regions that have been subject to selection in different populations.

CYTOGENETIC ANALYSIS IN PREMATURE OVARIAN FAILURE. *D. Pathak[1], R. Kumar[1], A. Sharma[1], A. Kriplani[2], AC. Ammini[3], R. Dada[1]* 1) Dept Anatomy, All India Inst Medical Sci, New Delhi, India; 2) Dept Obstetrics and Gyneocology,All India Inst Medical Sci, New Delhi, India; 3) Dept Endocrinolgy ,All India Inst Medical Sci, New Delhi, India.

Premature ovarian failure is premature cessation of menses before 40 years of age. The condition is characterized by primary amenorrhea, gonadal dysgenesis and elevated gonadotrophin levels. The aetiology of POF is complex and several causes like auto antibodies, chemotherapy, autoimmune disease and diabetes mellitus are associated with POF. In order to study genetic aberrations in idiopathic POF, conventional cytogenetics of 50 idiopathic POF cases was performed, after informed consent and ethical clearance. Chromosome preparations were obtained by 72 hour peripheral blood culture by GTG banding. 39 cases revealed a normal 46, XX karyotype and 11 cases revealed an abnormal karyotype. Out of 11 cases 6 cases are mosaic with 45 ,XO, 46, XX chromosomal complement. In 2 cases there were structural defects in X chromosome, where as in one case had 46, XX q del (13.3-Xq21.1) [60%] karyotype and one case had 46, X iso Xq [90%], 46 XX [10%]chromosomal complement. One case revealed 46, XY chromosomal complement with pure gonadal dysgenesis, PCR for SRY did not show amplification. This case had complete AZF deletion. In 2 cases there were structural autosomal defects , where as one case had 45, XO [25%]/ 46, X ring X [75%] and [30%] of cell line also showed 46 XX iso 2q chromosomal complement and One case had 46, XX[80%], 46 XX iso 22q[20%]. The results of this preliminary study showed the association of chromosome abnormalities in aetiology and pathogenesis of POF. Thus genetic study must be included in evaluation of idiopathic POF cases and cases with genetic abnormalities should be counselled comprehensively.

A Piece in the Puzzle of the INSIG2 rs7566605 SNP Association With Obesity Based on a Meta-Analysis Including 76,548 Individuals: It is the Study Population That Matters! *I. M. Heid[1], C. Huth[1], R. J. F. Loos[2], F. Kronenberg[3], B. Langer[1], C. Lange[4], N. Laird[4], H. E. Wichmann[1], INSIG2 Meta-Analysis Group* 1) Helmholtz Zentrum München, Neuherberg, Germany; 2) MRC Epidemiology Unit, Cambridge, UK; 3) Innsbruck Medical University, Austria; 4) Harvard School of Public Health, Boston, USA.

The INSIG2 rs7566605 SNP was identified to be associated with obesity (body-mass-index, BMI 30kg/m^2) in one of the first genome-wide association studies. Subsequent replication was shown in many, but not in all studies. We thus performed a meta-analysis to clarify the association and to explore potential sources of heterogeneity. We collected association statistics (recessive model) from general population based studies (GP, n=16), studies selected from a healthy population (HP, n=5), or case-control studies on extreme obesity (OB, n=6) involving 76,548 participant. We found no heterogeneity among GP studies (n=48,844, I^2=11%, p=0.329). Adding HP or OB introduced heterogeneity (I^2=44%, p=0.008). Thus, we conducted separate analyses for GP, HP and OB. The GP meta-analysis suggested increased obesity risk for the CC-homozygotes (OR=1.10, p=0.015) with increasing ORs for more extreme degrees of obesity: 1.20 (BMI 32.5kg/m^2), 1.26 (BMI 35.0kg/m^2), 1.31 (BMI 37.5kg/m^2), 1.41 (BMI 40.0kg/m^2) compared to <25kg/m^2 (p-values between 0.048 and 0.0002). Two out of 3 pre-defined subgroups indicated significant heterogeneity: studies from more obese populations (more than average proportion of obese) and studies with obesity assessed more recently (after year 2000) showed higher associations than the less obese or less recent studies. In the OB studies, a consistent association between the rs7566605 and extreme obesity was observed (OR=1.16, p=0.038). No association was found in HP meta-analysis. In one of the largest genetic studies on obesity, we conclude that there is evidence for association of the INSIG2 rs7566605 CC genotype with increased obesity, which is most pronounced when using more extreme comparisons. Our data also suggests heterogeneous effects when pooling different study populations, which may mask underlying associations.

Dymeclin, the protein defective in Dyggve-Melchior-Clausen syndrome is a peripheral membrane protein dynamically associated with the Golgi apparatus. *A. Dimitrov[3], V. Paupe[1,2], C. Gueudry[3], J. B. Sibarita[3], G. Raposo[3], O. Vielemeyer[3], Z. Csaba[4], T. Attie-Bitach[2], P. Gressens[1], P. Rustin[1], F. Perez[3], V. El Ghouzzi[1,2]* 1) INSERM U676, hôpital Robert Debré, Paris, France; 2) INSERM U393, hôpital Necker, Paris, France; 3) UMR CNRS 144, Institut Curie, Paris, France; 4) INSERM U686, Université Paris Descartes, Paris, France.

Dyggve-Melchior-Clausen (DMC) syndrome is a rare inherited dwarfism with severe mental retardation resulting from loss-of-function mutations in the Dym gene which encodes Dymeclin, a 669-aminoacid protein of yet unknown function. Despite a high conservation across species and several predicted transmembrane domains, Dymeclin could not be ascribed to any family of proteins. To obtain additional clues on Dymeclin function, we studied the expression pattern of Dym in human embryo-foetal tissues using in situ hybridization and we used the recombinant protein fused to GFP to investigate its sub-cellular localization. The transcript is widely expressed in human embryos, especially in the cortex, the hippocampus and the cerebellum and that the protein co-localizes with Golgi apparatus markers. Electron microscopy revealed that Dymeclin associates with the Golgi apparatus and with transitional vesicles of the reticulum-Golgi interface. Dymeclin is myristoylated in vitro but the myristoylation is not essential for Golgi localization. Permeabilization assays further revealed that Dymeclin is a peripheral protein of the Golgi apparatus as it can be completely released from the Golgi after permeabilization of the plasma membrane. Moreover Fluorescence Recovery After Photobleaching experiments have shown that the half time of recovery is as short as 2.8 seconds for the entire Golgi apparatus. Interestingly, in the presence of nocodazole which depolymerizes microtubules, Dymeclin did not associate with mini-stacks but only with membranes of the old, pre-existing, Golgi apparatus, in a highly dynamic manner. These results indicate that Dymeclin recognizes specifically a subset of mature Golgi apparatus and shuttles extremely rapidly between the cytosol and these membranes, and suggest that DMC results from an impairment in Golgi/ER membrane trafficking .

Allelic background of 9p21 loci associated with type 2 diabetes and coronary heart disease in 51 global populations. *K. Silander[1], S. Myles[2], H. Tang[3], E. Jakkula[1], L. Cavalli-Sforza[3], N. Timpson[4,5], L. Peltonen[1,4]* 1) Institute of Molecular Medicine and NPHI, Helsinki, Finland; 2) Institute for Genomic Diversity, Cornell University, Ithaca, NY, USA; 3) Stanford University School of Medicine, CA, USA; 4) Wellcome Trust Centre for Human Genetics, Oxford, UK; 5) MRC CAiTE Centre, University of Bristol, UK.

A 100kb region on 9p21.3 harbors two major disease susceptibility loci: one for type 2 diabetes (T2D) and one for coronary heart disease (CHD). The SNPs associated in Europeans reside on two adjacent haplotype blocks having independent effect on disease. HapMap phase 2 data suggests that identification of functional variants and selection of the most relevant SNPs for studies in global populations will be challenging in this region due to the diverse LD and haploblock structure in different populations. We analyzed the allelic background of this region in 938 unrelated individuals from 51 populations (HGD panel). We used the Illumina 650Y SNP dataset (Li et al. 2008), supplemented with 5 additional critical SNPs. The T2D risk allele tagged by rs10811661 was found on wide diversity of haplotype backgrounds, whereas a short (5.5 kb) shared haplotype was tagged by the protective allele of this SNP (0.03 in Africa, 0.16 in Europe and 0.27 in Asia). This 5.5kb region and even this particular SNP might represent the actual functional domain. Opposite to T2D locus, for the CHD locus all populations shared a core risk haplotype spanning 30 kb, tagged by the risk allele of rs4977574 (0.11 in Africa, 0.65 in Middle East, 0.57 in Europe, 0.51 in Asia). Interestingly, the SNP most strongly associated with CHD, rs10757278, is in perfect LD with rs4977574 25kb upstream in the YRI HapMap population, suggesting these SNPs would probably be the most appropriate for further analyses in non-Europeans. Our results demonstrate the importance of allelic background data when selecting SNPs for replication in global populations. Intriguingly, these initial data also imply increase in prevalence of T2D protective allele versus increase of CHD risk allele in out of Africa populations implying different population histories for these two adjacent disease loci.

***homruns*: A graphical tool to locate and visualize runs of homozygosity in whole-genome SNP data.** *R. Karlsson, S. Paddock* Department of Neuroscience, Karolinska institutet, Stockholm, Sweden.

We present the cross-platform software package *homruns* for finding and visualizing runs of homozygosity in whole-genome SNP datasets.

The interest for examining the role of copy number variations in human genetics has been increasing lately. One way to search for these relatively hard-to-get polymorphisms is to screen whole-genome SNP datasets for genotype patterns indicative of copy number variation, such as runs of homozygosity. Many such datasets are already available with even more on their way. In order to identify runs of homozygosity, one searches for coherent runs of homozygous markers. Such runs, if long enough that the likelihood of all markers being homozygous by chance becomes vanishingly small, can be an indicator of a deletion event, which would give false homozygous signals from a part of the genome that is hemizygous. In addition to detecting deletions, the same pattern of homozygosity can also be seen as the effect of a recent recessive mutation.

homruns uses a sliding window to calculate the per marker likelihood of being part of a homozygous region. Coherent stretches of markers with a high likelihood for being in such a region are then identified and displayed as a graph in the user interface, as well as saved in a plain text file for further analyses.

Parameters such as window size and the threshold for including a marker in a homozygous run can easily be adjusted by the user. The main calculation uses the *plink* toolset (Purcell et al, 2007; http://pngu.mgh.harvard.edu/purcell/plink/), which means that input data can be in any format readable by *plink*. The software program *homruns* is released under the GPL, and can be downloaded from the author's website for free.

Williams-Beuren Syndrome TRIM50 gene defines highly dynamic and mobile cytoplasmic bodies that localize with autophagosomes LC3 marker. *G. Merla[1], C. Fusco[1], M. Egorov[2], L. Micale[1], P. Turturo[1], B. Augello[1], M. Monti[3], R. S. Polishchuk[2], P. Pucci[3], F. Cozzolino[3], A. Reymond[4]* 1) Medical Genetics Unit, IRCCS Casa Sollievo della Sofferenza, San Giovanni Rotondo, Italy; 2) Unit of Membrane Sorting and Biogenesis Department of Cell Biology and Oncology, "Mario Negri Sud Consortium" Santa Maria Imbaro, Italy; 3) CEINGE Advanced Biotechnology and Department of Organic Chemistry and Biochemistry, Federico II University, Naples, Italy; 4) Center for Integrative Genomics, University of Lausanne, Lausanne, Switzerland.

We recently showed that the protein encoded by the TRIM50 gene was part of the E3-ubiquitin ligase family that catalyze the transfer of ubiquitin moieties to specific substrates. These post-translational modification either contribute to the fine regulation of the substrate or to its tagging for degradation by the proteasome. TRIM50 is hemizygous in the Williams Beuren Syndrome (WBS), a contiguous genetic disorder characterized by a number of physical and developmental disabilities. WBS is caused by a 1.5 Mb deletion at 7q11.23 that includes at least 25 genes. To get insight on the role of TRIM50 we performed fluorescence and electronic microscopy to study the TRIM50 cellular localization and we used functional proteomic strategies to identify proteins interacting with this E3-ubiquitin ligase. We found that the TRIM50 protein localizes in highly mobile, labile and dynamic cytoplasmic bodies, it is capable of assembling larger cytoplasmic bodies from several individual smaller particles and importantly it colocalizes with LC3, a specific marker of autophagosomes. Consistently, nano LC MS/MS identified a number of putative TRIM50 interacting proteins known to be implicated in the proteasome and autophagy pathways. These results show an unexpected role for TRIM50 on protein degradation pathways. We speculate that the haploinsufficiency of TRIM50 could account for a consistent part of WBS phenotype through the accumulation and/or abnormal degradation of TRIM50 substrates.

Activating FGFR3 mutation cause hearing loss and inner ear defect in a new mouse model of thanatophoric dysplasia. *S. Pannier[1], V. Couloigner[2], N. Messadeq[3], M. Elmaleh-Bergès[4], A. Munnich[1], R. Romand[3], L. Legeai-Mallet[1]* 1) INSERM U781, Hopital necker, Paris, France; 2) Service Oto-Rhino-Laryngologie-Chirurgie Cervico faciale-Hopital Necker, Paris, France; 3) IGBMC, Illkirch, France; 4) Service d'imagerie pédiatrique, Hôpital Robert Debré, Paris, France.

Fibroblast growth factor receptor 3 (FGFR3) is a key regulator of skeletal development and activating mutation in FGFR3 cause several skeletal dysplasias, including hypochondroplasia, achondroplasia and thanatophoric dysplasia. Here, we described a new mouse model for thanatophoric dysplasia. We introduced the Y367C mutation corresponding to the human TDI mutation (Y373C) into the mouse genome. The mice displayed dwarfism with a skeletal phenotype remarkably similar to that of human chondrodysplasia. To investigate the role of activating FGFR3 mutation in auditory function, we examined heterozygous mutant $fgfr3^{Y367C/+}$ mice. We show here, for the first time, that mutant $fgfr3^{Y367C/+}$ mice exhibit mild deafness with a significantly elevated ABR threshold for all frequencies tested. A severe ossification delay in the cochlea and the ossicular chain is observed in the mutant mice. The inner ear defect is mainly associated with an increased number of pillar cells or modified supporting cells in the organ of Corti. Hearing loss in fgfr3 mouse model confirms the crucial role of FGFR3 in the development of the inner ear and provides novel insight on the biological consequences of FGFR3 mutations.

CYTOGENETIC ASPECTS OF MYELODYSPLASTIC SYNDROMES. *R. kumar[1], D. Pathak[1], R. Chaubey[2], p. Tiwari[2], R. Saxena[2], R. Dada[1]* 1) Deptt ofAantomy, AIIMS, Delhi, India; 2) Deptt of Hematology,AIIMS,Delhi,India.

Myelodysplastic Syndrome (MDS) is a clonal disorder of haematopoietic stem cells and results in progressive cytopenia and defect in erythroid, myeloid and megakaryocytic maturation. The diagnosis of MDS is difficult to establish based on morphological features alone because dysplasia is not always be detectable and the presence of dysplasia is not itself evidence of clonal disorder. As a result, the detection of clonal cytogenetic abnormality has a major role in diagnosis and classification of MDS and determining its prognosis. In an attempt to assess the frequency and characteristics type of abnormal clones, cytogenetic analysis was carried in 25 MDS cases. Cytogenetic analysis of bone marrow cells and peripheral blood cells was carried by 24 hour unstimulated cell culture and 72 hour stimulated blood culture respectively. Chromosomes were obtained by GTG bands. On analysis 12 out of 25 patients had abnormal karyotypes. In 8 patients cytogenetic changes were characteristic of those reported for MDS: del(5q)[n=2], monosomy [n=3], monosomy 7[n=2] and 1 had Y chromosome loss. Out of 12 cases with cytogenetic abnormalities 4 cases had cytogenetic abnormalities which have not been previously reported in MDS cases. Out of these cases trisomy 9, t(12q-6q), iso(9q) and t(12p-6q) were found in each case each. Long term follow-up and larger studies of such cases are required to determine the malignant potential of these clones and prognosis in MDS cases.

Absence of coding region mutations in predicted mitochondrial proteins within the X-chromosomal candidate interval linked to visual failure in Leber hereditary optic neuropathy. *P. Yu Wai Man, G. Hudson, P. F. Chinnery*
Newcastle University, Newcastle Upon Tyne.

Leber hereditary optic neuropathy (LHON, OMIM 535000) is an early onset (median = 24yrs) bilateral visual failure, resulting from retinal ganglion cell loss, which predominantly affects males (4:1). 95% of all LHON is caused by one of three mtDNA point mutations in genes which encode complex I subunits; 3460G>A in *MTND1*, 11778G>A in *MTND4* and 14484T>C in *MTND6* . However, mtDNA mutations cannot explain why only ~50% of male and ~10% of female carriers develop symptoms. This incomplete penetrance indicates the influence of epigenetic factors on the onset of symptoms. Until recently attempts to map the locus were inconclusive. We recently identified a 60cM region of the X-chromosome (Xp21.1 - Xq21.1. In recent years proteomic based approaches have been developed to assist the determination of nuclear encoded mitochondrial proteins and Integrative genomics has been applied to the identification of two nuclear encoded mitochondrial disease genes; *LRPPRC*. We replicated this strategy to attempt to identify the nuclear encoded mitochondrial protein thought to modulate the onset of LHON. Mitochondrial prediction tools (*Masetro*, *MitopII*, *Mitoprot*, *TargetP*, *WolfPsort*) were used to construct a candidate gene list which could be screened in a panel of patients. Our bioinformatic analysis identified 25 candidate genes, subsequently screened in 8 discordant siblings. We identified 24 single nucleotide polymorphisms in 11 of the candidate genes. One synonymous single nucleotide polymorphism was found in LD (1350T>C, P=0.0067) between cases and controls. However, screening of the remaining indentified SNPs in a larger patient cohort revealed no significant difference to controls. No significance was found when comparing LD or when constructing complex genotypes. Although we have sequenced the entire coding regions of 25 genes, we cannot rule out the possibility of a non-coding, UTR or promoter region change which may modulate the phenotype of LHON. In conclusion, previously successful bioinformatic tools have failed to identify the LHON nuclear modifying locus.

Allelic alterations in epithelial ovarian carcinomas uncovered by Affymetrix SNP Array 5.0. *A. Tajima[1], K. Yoshihara[2], I. Inoue[1], K. Tanaka[2]* 1) Dep Molecular Life Sci, Tokai Univ Sch Medicine, Kanagawa, Japan; 2) Dep Obstet & Gynecol, Niigata Univ Graduate Sch Med & Dent Sciences, Niigata, Japan.

Ovarian cancer is the most lethal form of gynecological malignancy. Complex cytogenetic alterations due to chromosomal instability are regarded as an underlying mechanism of ovarian carcinogenesis. Many previous studies have contributed to the identification of recurrent changes in genomic copy number and loss of heterozygosity (LOH) in ovarian cancers. Despite such knowledge, however, genetic factors underlying the changes remain poorly understood. To further extend our knowledge of genomic instability in ovarian cancers, we here attempt to make a high-resolution map of cytogenetic aberrations using Affymetrix SNP Array 5.0, featuring over 920,000 genetic markers (500,000 SNPs and 420,000 non-polymorphic probes) for the measurement of genetic differences. To obtain a comprehensive catalog of copy number alterations, we analyzed matched tumor and non-tumor genomes from 43 patients with epithelial ovarian cancers (33 sporadic; 10 familial with *BRCA1* germline mutations). Partek Genomics Suite software was used to identify regions of copy number gain and loss, and estimate the copy number. The number of somatic copy number changes per cancer genome and the relative proportion of gains to losses varied between tumors, indicating cytogenetic heterogeneity in ovarian carcinomas. Of note, monoallelic alterations accounted for the vast majority of the observed copy number changes. These features were commonly observed in two groups of sporadic and *BRCA1*-associated tumors. Allele-specific gains were frequently observed on 3q, 5p, 7q, 8q and 20p, as were losses on 4q, 5q, 17p and 18q among the 43 tumors examined. We will discuss the implications in carcinogenesis, combining with our recent findings from genome-wide gene expression analysis.

Analysis of epidermal stem cells in a mouse model of Hutchinson-Gilford Progeria Syndrome. *Y. Rosengardten[1], H. Sagelius[1], T. McKenna[1], B. Rozell[2], M. Eriksson[1]* 1) Department of Biosciences and Nutrition, Karolinska Institutet, Karolinska University Hospital, Huddinge, Novum, SE-141 86 Stockholm, Sweden; 2) Clinical Research Center, Department of Laboratory Medicine, Karolinska Institutet, Karolinska University Hospital, Huddinge SE-141 86 Stockholm, Sweden.

Hutchinson-Gilford progeria syndrome (HGPS) is an extremely rare genetic disorder characterized by symptoms suggestive of accelerated aging. Most cases of HGPS are due to a *de novo* single point mutation in exon 11 of the *LMNA* gene, 1824CT (G608G). The *LMNA* gene encodes the A-type lamins, which are major proteins of the inner nuclear lamina. The HGPS mutation activates a cryptic splice site resulting in a truncated lamin A protein, progerin. In this study we have utilized our previously published transgenic mouse model with epidermal expression of the HGPS mutation that replicates several features of the HGPS skin phenotype. Histopathological examination of these mice showed a progressive phenotype with early stages characterized by regions of epidermal hyperplasia, and hyperproliferative epidermal cells, and later stages characterized by epidermal hypoplasia, hypoplastic sebaceous glands, loss of hypodermis and a fibrotic dermis (JCS 121, 969-978, 2008). In this study we have used FACS analysis with antibodies directed against CD34 and 6-integrin on keratinocytes isolated from these mice. CD34 and 6-integrin are commonly used markers for keratinocytes with stem and progenitor cell characteristics. Our results showed that within 27 weeks of transgenic expression, there was a decrease in the 6-integrin/CD34 positive cell population compared to wildtype mice. Additional results from label-retaining cell analysis and colony-forming assays on epidermal stem cells in our HGPS model will be presented.

Kidney and muscle phenotypes due to hyposialylation in a mouse model of Hereditary Inclusion Body Myopathy.

M. Ziats[1], D. Kurland[1], D. Hickey[1], R. Klootwijk[1], I. Manoli[1,2], K. Patzel[1], C. Ciccone[1], P. Zerfas[3], M. Starost[3], D. Darvish[4], WA. Gahl[1,2], M. Huizing[1] 1) MGB, NHGRI, NIH; 2) Office of Rare Diseases, OD, NIH; 3) Div Vet Res, NIH, Bethesda, MD; 4) HIBM Research Group, Encino, CA.

Hereditary Inclusion Body Myopathy (HIBM) is a recessive adult-onset neuromuscular disorder, characterized by progressive muscle weakness due to mutations in UDP-GlcNAc 2-epimerase/ManNAc kinase (GNE), the key enzyme in sialic acid (SA) synthesis. We created a *Gne* knock-in mouse model harboring the human M712T mutation. We previously showed that these mice died before postnatal day 3 (P3) of glomerular disease, involving effacement of podocytes due to hyposialylation. Administration of the SA precursor ManNAc partially rescued the kidney phenotype and allowed survival of mutant mice. Here we evaluate SA itself as a therapeutic agent, by oral administration to pregnant and nursing mice. SA feeding did not significantly increase the number of surviving mice beyond P3, likely due to the negative charge of SA (impairing transmembrane transport) compared to the neutral charge of ManNAc. We also evaluated the evidence of a muscle phenotype in older surviving mutant mice. Electron microscopy studies of the gastrocnemius, gluteus, and quadriceps muscles of 6 and 11 month old mutant mice showed tubular aggregates (TAs). TAs presumably originate from the sarcoplasmic reticulum, and may be precursors of the rimmed vacuoles (RVs) seen in the muscles of HIBM patients, who are diagnosed late, after RVs have already formed. Further analysis of TA formation and sialylation status of affected muscles is being pursued, as well as evaluation of the effect of ManNAc on their formation. Other human disorders characterized by TAs, including sporadic limb girdle weakness, familial myasthenia gravis, and unexplained exercise-induced muscle cramps, may be caused by local sialic acid deficiency. Our *Gne* M712T mouse is a model for further evaluation of these hypotheses. In sum, our *Gne* M712T mouse unexpectedly serves as the first genetic model of podocyte injury due to hyposialylation, and may also prove to be a model of the myopathy of HIBM.

No significant association of 768 markers in 68 candidate genes with major depression in the STAR*D cohort. *M. Lekman[1], D. Charney[2], H. Manji[3], A. J. Rush[4], F. J. McMahon[3], S. Paddock[1]* 1) Department of Neuroscience, Karolinska institutet, Stockholm, Sweden; 2) Mount Sinai School of Medicine, NY, NY; 3) NIMH, NIH, Bethesda, MD; 4) University of Texas, Dallas, TX.

Major depressive disorder (MDD) is a highly debilitating disorder affecting a large proportion of the global population. So far, no genetic predictor for the disorder has been identified consistently, although a genetic predisposing factor has been reported in many epidemiologic studies. Large sample sizes are needed in order to identify genetic contributors for disease with only modest effect. Therefore, the large DNA sample available from the STAR*D study (N=1953) provides a good chance to elucidate genetic factors of small effect. Included individuals were between 18-75 years of age, diagnosed according to the DSM-IV criteria as non-psychotic MDD, and had a baseline score on the Hamilton Rating Scale for Depression HAM-D17 14. A sample of 1256 affected individuals of White Caucasian ancestry was matched with 634 controls. The control sample DNA was obtained from the NIMH Genetic Initiative for schizophrenia. All control samples were screened (DSM-IV) for not being affected by either major depression, bipolar disorder or psychosis. Markers (SNPs) were located in 68 genes previously implicated in the pathogenesis of major depressive disorder (for a complete list of all markers see McMahon et al. Am J Hum Genet. 2006 May;78(5):804-14.). Genotyping was carried out by Illumina with a success rate of 99.78%. The cocaphase software program was used in order to test for allelic association of each marker with disease status. A total of 47 SNPs (6% of the total markers) showed nominally (p0.05) significant p-values, located on chromosomes 3, 4, 5, 6, 9, 11, 12, 13, 16, 18, 21 and X. The most significant p-value (p=0.00031) was found at a marker nearby the GRIA3-gene on the X-chromosome. However, after correction for multiple testing, none of the associations remained significant. In an ongoing study, we now aim to replicate the nominally significant associations using the even larger GAIN case-control cohort for MDD.

Isolated CDH1 germline small deletion in a family with early onset breast cancer detected by Array-CGH. *C. Palka[1], G. Calabrese[1,2,3], A. Novelli[4], E. Morizio[3], P. Guanciali Franchi[1,3], C. Nuzzi[3], G. Palka[1,3], B. Dallapiccola[4]* 1) Biomed Science Dept./Medical Genetics, University G. DAnnunzio, Chieti, Italy; 2) Center for Ageing, DAnnunzio Foundation, Chieti, Italy; 3) Medical Genetics "Spirito Santo" Hospital, Pescara, Italy; 4) CSS-Mendel Institute, Rome, Italy.

We evaluated a 3,5-year-old boy because of psychomotor delay and craniofacial dysmorphisms. Probands standard karyotype resulted as normal 46,XY. Therefore, we performed an array-CGH analysis, with a 75Kb resolution, which disclosed a microdeletion of 0.24Mb on chromosome 16q22.1. The chromosomal abnormality was confirmed with FISH analysis. Parents investigation demonstrated that his mother had the same alteration. This deletion encompasses a small chromosomal region which includes few genes, such as ZFP90, cerebrally expressed, and CDH1, coding for E-cadherin, involved in pathogenesis of invasive lobular carcinoma of the breast. Probands family history was positive for tumours. In particular, his mother had a right invasive lobular breast carcinoma combined with a carcinoma in situ, both surgically removed when she was 35-year-old. She was negative for germline BRCA1 and BRCA2 mutations. Proband's maternal grandfather died for right colon cancer, while his great-grandmother from maternal line died for breast cancer. To our knowledge this is the first case of breast lobular carcinoma harbouring germline, isolated CDH1 partial deletion without involvement of other tumor-related flanking genes previously reported as rearranged together with CDH1 within 16q22 in breast lobular cancer. Fine molecular characterization of 16q22 deletion region is currently underway, also including uniparental disomy analysis to verify possible correlation between proband's phenotype and chromosome 16q22, a known imprinted region containing a number of genes or putative genes expressed in brain.

ABL-DEPENDENT PHOSPHORYLATION OF MEGAP/SRGAP3 AFFECTS ITS BINDING TO WAVE1. *G. Rappold, V. Endris, L. Hausmann* Dept. of Human Molecular Genetics, University of Heidelberg, Heidelberg, Germany.

Mental retardation affects up to two percent of the population and is accompanied by impaired learning and memory. Loss of function mutations of the MEGAP/SrGAP3 (Mental disorder associated GAP protein) gene has been previously described in patients with mental retardation, ataxia and complete lack of speech (Endris et al, 2002). MEGAP/srGAP3 is a member of the Slit-Robo GAP family which is involved in the transduction of repulsive guidance signals. Slit/Robo signaling plays an important role in the migration of neuronal precursor cells and axon guidance during the development of the nervous system. MEGAP represents a Rac1-specific GTPase activating protein, which binds to and inhibits the functions of the WASP related protein WAVE1. Here, we report on the regulation of MEGAP by c-Abl. Protein binding analysis revealed that MEGAP interacts with c-Abl through a region distal to its SH3 domain. We identified two MEGAP phosphorylation sites, one located within the Abl-binding interval (Y845) and one within the SH3 motif (Y755). Phosphorylation of Y755 leads to a charge change that abolishes the binding of MEGAP to WAVE1, a key regulator of actin-dependent morphological processes. Our results suggest that c-Abl regulates the function of MEGAP via phosphorylation, thereby uncoupling the inhibitory effect of MEGAP on the WAVE1 complex.

Genotype-phenotype analysis in patients with the L1 syndrome. *Y. J. Vos, J. A. Stegeman, H. E. K. de Walle, I. Stolte-Dijkstra, R. M. W. Hofstra* Dept of Genetics, University Medical Centre, Groningen, The Netherlands.

The L1 syndrome is an X-linked recessive disorder with an incidence of one in every 30,000 newborn males. It comprises four neurological syndromes, all being found associated with mutations in the *L1CAM* gene. These syndromes being: HSAS (X-linked Hydrocephalus with Stenosis of the Aqueduct of Sylvius), MASA (Mental retardation, Aphasia, Spastic paraplegia and Adducted thumbs), SPG1 (X-linked complicated hereditary spastic paraplegia type 1) and X-linked ACC (Agenesis of Corpus Callosum). In our laboratory an efficient mutation screening method has been developed and used to analyse the DNA of more than 300 patients. As a result, a pathogenic mutation has been found in only 25% of all L1 syndrome patients, which is a rather low score. Since the costs of mutation analysis are relatively high, a study has been undertaken to define (clinical) criteria which would able us to increase the efficiency in actually finding a relevant mutation, i.e. a higher detection rate. As a result we found that in families with more than one affected family member, the detection rate is about 60%. In patients with three or more clinical characteristics, belonging to the L1 syndrome, the detection rate is about 65%. Combining these two, i.e. patients having a positive family history and having more than three characteristics of the L1 syndrome, the detection rate is almost 85%, all findings being statistically significant. These calculations are based on the identification of both truncation mutations and missense mutations. We also looked for possible correlation between the seriousness of the disease and the type of mutation found. No statistically significant differences were detected in the seriousness of the disease in patients with a truncation mutation compared to patients with a missense mutation.

BAC Clone FISH approach to determine the breakpoint in the chromosomal rearrangement. *E. Shin[1], S. Li[2]* 1) Genome Research Center, NeoDin Medical Institute, Seoul, Korea; 2) Departments of Pediatrics, the University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104, United States of America.

Identification of chromosomal rearrangements involving derivative chromosomes was considered as difficult and sometimes impossible with classical cytogenetic banding methods. The determination of their chromosomal breakpoint is now easier with fluorescent in situ hybridization (FISH) techniques using BAC Clone and enables an exact correlation between chromosomal aberration and phenotypic features to be established. FISH using BAC Clone was performed to define the breakpoints involved in the formation of der(10), der(15) and der(X). Molecular cytogenetic analyses of the chromosome breakpoints revealed the localization of the breakpoint in 10q26 between BACs RP11-113M14, RP11-7P17 and RP11-184L4, each BACs harbour the OAT gene, the FGFR2 gene and the DOCK1 gene, respectively. In 15q21-26, a breakpoint-spanning clones (RP11-243M9 and RP11-289H7) were localized which contain the genes THSD4 and ETFA. On chromosome Y, BACs RP11-49G16 and RP11-1036K16 were used to confirm the genes TSPY1 and RBMY1A1 on chromosome der(X)t(X;Y), respectively. In conclusion, these studies identified der(10) case as 46,XX,der(10)dup(10q26).ish del(10)(qter-)(OATX2,FGFR2X2,DOCK1X2), der(15) case was also characterized as 46,XY,der(15)del(15)(q26)dup(15)(q26q21).ish der(15)(PML++,15qter-,THSD4X2,ETFAX2) and 46,X,der(X)t(X;Y) with the presence of the YRRM and TSPY gene to help the understanding the clinical phenotype of each case by breakpoint analyses.

***POLG1* variants and sporadic idiopathic Parkinsons disease.** *D. Burn[1], G. Hudson[2], A. Stutt[1], M. Eccles[3], L. Robinson[3], P. F. Chinnery[1]* 1) Institute for Ageing and Health, Newcastle University, UK; 2) Mitochondrial Research Group, Newcastle University, UK; 3) Institute for Health and Society, Newcastle University, UK.

The prevalence of Parkinsons disease is estimated at ~0.3% of the population. Clinically, PD is homogenous however PD genetics are heterogeneous. PD aetiology is complex, and likely multifactorial, with several biochemical abnormalities relevant to disease onset; including mitochondrial dysfunction, reinforced by the identification of a complex I deficiency in PD brains. Mitochondrial DNA polymerase gamma (*POLG1*) is a nuclear encoded gene required for the synthesis and replication of the mitochondrial genome. Mutations in the gene encoding polg have been reported in a wide range of clinical phenotypes. Genetic variation in *POLG1* could contribute to the etiology of parkinsonism in a number of ways. The absence of a clear family history is common in late-onset medelian disorders, raising the possibility that dominant *POLG1* mutations are responsible for sporadic parkinsonism. Secondly, common POLG1 variants affect polymerase activity, and thus might contribute to the risk of developing sporadic idiopathic PD. We compared 7 previously investigated common POLG1 variants (c.722C>T - P241L; c1174C>G - L392V; c.2165G>A - R722H; c.2492A>G - Y381C and c.3689T>C - S1230F), in addition to seven common alleles, previously described in the UK population, (c.1399G>A- A467T; c.2209G>C - G737R; c.2243G>C - W748S; c.2557C>T - R853W; c.2864A>G - Y955C; c.3248G>A - E1143G and c.3708G>T - Q1236H), as well as the *POLG1* CAG repeat, in 221 community based UK-PD cases to 159 ethnically, gender and age matched controls. Our analysis indicates that common variants within *POLG1* do not contribute to the etiology of PD. We found no association between individual alleles, compound genotypes or the number of *POLG1* substitutions in cases and controls. Rare (CAG)n repeat lengths have been previously associated with, however we found no evidence that (CAG)n repeat lengths are associated with U.K. PD. We conclude that it is unlikely that common genetic variation in *POLG1* is a major contributor to sporadic idiopathic PD.

Histone H3 lysine 27 trimethylation in adult differentiated tissue associated to cancer DNA hypermethylation. *C. Wadelius[1], A. Rada-Iglesias[1, 2], S. Enroth[2], R. Andersson[2], A. Wanders[1], L. Påhlman[3], J. Komorowski[2, 4]* 1) Dept Genetics & Pathology, Uppsala Univ, Uppsala, Sweden; 2) Linnaeus Centre for Bioinformatics, Uppsala University, SE-75124 Uppsala, Sweden; 3) Department of Surgical Sciences, Uppsala University Hospital, SE-75185 Uppsala, Sweden; 4) Interdisciplinary Centre for Mathematical and Computer Modelling, Warsaw University, Poland.

DNA methylation at promoters is a common epigenetic abnormality in cancer which is associated with silencing of the genes. Several recent reports have suggested that the instruction for this methylation is present already in embryonal stem (ES) cells in the form of another epigenetic mark, namely trimethylation of histone 3 at lysine 27 (H3K27me3). We used chromatin immunoprecipitation and detection on promoter arrays (ChIP-chip) to generate H3K27me3 and H3K4me3 profiles for normal colon mucosa from a patient with colon cancer and compared them to similar profiles for ES cells. There was a considerable overlap between genes with H3K27me3 modification in normal colon and ES cells ($p=0$). At the same time the cell identity is present in the histone profiles in colon since the genes marked by H3K4me3 are on average highly to moderately expressed whereas the genes with the H3K27me3 modification are inactive, consistent with previous data. By comparing to a set of genes that are DNA methylated in colon and/or prostate cancer, we found that a significant number of them are marked by H3K27me3 in both normal colon and ES cells. Genes marked by H3K27me3 in ES cells and normal colon are both overrepresented among genes that are DNA-methylated in five common cancers. When dividing 68 genes that are DNA methylated in one or more of these cancers in methylated/unmethylated for each cancer, the H3K27me3 status in normal colon was a good predictor of DNA-methylation in colon cancer ($p=.0003$) but not in other cancer types, whereas H3K27me3 in ES cells could not predict DNA-methylation in any cancer type. This suggests that H3K27me3 pre-marking of genes for cancer DNA hypermethylation is not necessarily restricted to ES or early precursor cells but can occur later in differentiated tissues.

SMS and PTLS mouse models to study effects of genome structural changes on expression. *G. N. S. Ricard[1], J. Chrast[1], N. Gheldof[1], J. Molina[2], S. Pradervand[1], J. R. Lupski[3], K. Walz[2], A. Reymond[1]* 1) Center for Integrative Genomics, Lausanne, Switzerland; 2) Centro de Estudios Cientificos, Valdivia, Chile; 3) Baylor College of Medicine, Houston, TX, USA.

To study the effect of structural changes on expression we assessed gene expression in genomic disorder mouse models. Both a microdeletion and its reciprocal microduplication mapping to the B2 region of murine chromosome 11, which model the rearrangements present in Smith-Magenis (SMS; MIM182290) and Potocki-Lupski (PTLS; MIM610883) syndromes patients, respectively, have been engineered. These models show phenotypic features similar to those identified in human SMS and PTLS patients. Using Affymetrix arrays, we profiled the transcriptome of five different tissues affected in human patients in mice with 1n (Deletion/+), 2n (wild type; +/+), 3n (Duplication/+) and 2n on a single allele (Deletion/Duplication) copies of the same region in an identical genetic background. The most differentially expressed transcripts between the four studied genotypes were ranked. 23 to 28 of the top 100-ranked differentially expressed genes, a highly significant propensity, are mapping to the engineered SMS/PTLS interval in the different tissues. These genes are expressed on average 1.5 fold less and 1.35 fold more in the tissues of the mice carrying the deletion and the duplication, respectively. Interestingly, a statistically significant overrepresentation of the genes mapping to the flanks of the engineered interval was also found in the top 100 and top 500-ranked differentially expressed genes. An effect that extends over more than ten megabases from the breakpoints. Our results obtained on five different tissues suggest that this phenomenon is efficient across multiple cell lineages. We will also discuss the molecular networks that are altered in the different models. This network analysis enables the identification of metabolic pathways that potentially play a function in the SMS/PTLS phenotypes and allows a better comprehension of the roles of the different genes of the interval, in particular Rai1.

Expression of R1, R2 and p53R2 subunits of the ribonucleotide reductase in human and mouse tissues during the perinatal period. *A. Bourdon[1], F. Foufelle[2], I. Hainault[2], P. Ferré[2], A. Munnich[1], A. Rötig[1]* 1) INSERM U781, Hopital Necker, Paris, France; 2) INSERM UMRS872, Centre de Recherche des Cordeliers, Paris, France.

Mitochondrial DNA (mtDNA) depletion is a reduction in mtDNA copy number. We have identified mutations of the p53R2 gene encoding the cytosolic p53-inducible ribonucleotide reductase (RNR) small subunit in patients with mtDNA depletion, demonstrating how cytoplasmic dNTP pools are important for mtDNA replication. RNR is responsible for conversion of NDP into dNDP, essential for DNA synthesis. This enzyme consists of two R1 subunits and two R2 or p53R2 subunits. R2 is expressed only during the S phase but R1 is constantly expressed. p53R2 is present at low level during all the cell cycle. This implies that non proliferative tissues entirely rely on p53R2/R1 complex for dNDP synthesis and mtDNA replication. Patients with p53R2 mutations were normal at birth but then developed hypotonia and tubulopathy and present a severe mtDNA depletion in muscle. In order to investigate this tissue specificity we wonder how the three RNR subunits are regulated. We first observed that R1 and R2 are expressed at high level in various human fetal tissues. At 9 months of age, R1 showed a low expression level whereas R2 was almost undetectable. We have then followed the expression of R1, R2 and p53R2 from 14 days of prenatal development to 30 days after birth in tissues from control mice. We observed a constant and rapid decrease of R2 expression until day 8 after birth, whereas its expression became then very low in brain, liver, heart and kidney. R1 expression slowly decreased but remained significantly expressed after birth. On the contrary, p53R2 was constantly expressed in all tissues at all periods of time but significantly increased in liver and heart after birth. The regulation of RNR has been extensively studied during cell cycle but its expression during development has never been investigated. Our study shows that RNR subunits are differentially expressed in various organs in the perinatal period and this could partially been related to the organ involvement observed in patients with p53R2 mutations.

Genome-wide association study in 2,800 French identifies new susceptibility loci for extreme polygenic obesity. *D. Meyre[1], J. Delplanque[1], S. Lobbens[1], S. Gallina[1], C. Lecoeur[1], S. Visvikis-Siest[2], B. Balkau[3], A. Walley[4], C. Dina[1], P. Froguel[1,4]* 1) CNRS UMR 8090, Lille, France; 2) INSERM CIC 9501, Nancy, France; 3) INSERM U780-IFR69, Villejuif, France; 4) Section of Genomic Medicine, Imperial College, London, UK.

Recently, GWA studies have conferred to FTO and MC4R genes a convincing role in body weight variation. To identify additional susceptibility variants influencing risk of obesity, we analyzed genome wide association data (Illumina Human CNV370 duo) from 2,862 French individuals informative for body mass index (BMI). After discarding 62 individuals having less than 90% of European ancestry, we analyzed three affection binary traits (childhood obesity, adult obesity, pooled childhood / adult obesity) in 685 lean children (BMI Z score: -0.140.97, age: 11.932.27, male: 48.5%), 685 children with extreme familial obesity (BMI Z score: 4.281.24, age: 10.893.27, male: 45.3%), 731 lean adults (BMI: 21.771.87, age: 50.228.19, male: 24.8%) and 695 adults with morbid familial obesity (BMI:47.267.60 , age: 44.1311.97 , male: 21.03%), using both additive, dominant and recessive models. The best association signal was observed in the FTO intron 1 region for risk of pooled childhood and adult obesity under the additive model (rs8050136, P=3.10-13). The association upstream of MC4R gene was also confirmed in our study, again under the additive model (rs12970134, P=8.10-8). We identified 14 independent SNPs showing association with childhood obesity (N=3), adult obesity (N=7) or pooled childhood / adult obesity (N=4) at the genome wide level of significance (P<5.10-7). These SNPs were located in regions not previously associated with obesity. The genotyping of these SNPs is currently underway in 3,239 lean controls, 2,444 moderately obese and 3,178 extremely obese unrelated subjects, in 1,263 obesity-enriched pedigrees and in 10,489 population-based individuals (all of European ancestry), in order to confirm the associations found in WGA stage I. These results suggest that the study of extreme familial forms of disease is useful for the genetic dissection of complex traits.

Mutations of PEO1 gene encoding Twinkle helicase causes mitochondrial DNA depletion. *E. Sarzi[1], S. Goffart[2], D. Chrétien[1], V. Serre[1], A. Slama[3], A. Munnich[1], J. Spelbrink[2], A. Rötig[1]* 1) INSERM U781, Necker Hosp, Paris, France; 2) Institute of Medical Technology and Tampere University Hospital; 3) Laboratoire de Biochimie du Kremlin-Bicêtre.

Twinkle is a mitochondrial 5-3 DNA helicase is important for mitochondrial DNA (mtDNA) maintenance. Twinkle dominant mutations have been reported in progressive external ophthalmoplegia with multiple mtDNA deletions (adPEO) whereas Twinkle recessive mutations are associated with infantile onset spinocerebellar ataxia (IOSCA). It has been previously shown that Twinkle control mtDNA copy number renders Twinkle gene (PEO1) a candidate gene for mtDNA depletion. We selected a series of 10 patients born to consanguineous parents and presenting a severe mtDNA depletion of yet unknown origin. We then studied the segregation of microsatellite markers flanking PEO1 in these patients. Homozygosity of the microsatellite markers was found for two patients of the same family. They presented neonatal lactic acidosis, trunk hypotonia, seizures, cytolysis and cholestasis. A combined defect of complexes I, III and IV of the mitochondrial respiratory chain was found in liver of both patients as well as severe mtDNA depletion (8% of the normal mtDNA content). This prompted us to sequence PEO1 and to identify a homozygous mutation at a conserved position of the protein (T457I). The similarity of Twinkle and GP4D helicase from phage T7 prompted us to model the structure of this human helicase using the X-ray coordinates of the homohexameric GP4D protein as a tertiary template. Interestingly, the point mutation is located in the interface between two monomers of the hexameric enzyme, and can probably induce a local conformational change. During the in vitro validation, we have demonstrated that the T457I mutation leads to a decrease of the helicase activity in relation to a series of control. Then, we have shown that this mutation is responsible for a significant modification of the UTP Twinkle affinity. This work reports the first description of a PEO1 mutation responsible for mtDNA depletion in human.

Re-sequencing of the GRIK2 gene as a candidate gene for bipolar disorder and development of a software utility for search of interesting homozygous stretches in sequence data. *L. Hörnblad[1], R. Karlsson[1], F. J. McMahon[2], S. Paddock[1]* 1) Department of Neuroscience, Karolinska institutet, Stockholm, Sweden; 2) NIMH, NIH, Bethesda, MD.

Bipolar disorder is a severe mood disorder that affects about 1% of the adult population world wide. Symptoms include episodes of severe depression and mania with normal mood variability in between. Bipolar disorder has been shown to be highly heritable (Taylor L et al, 2002). The genetic background appears to be very complex with many different genes involved (Craddock and Jones, 1999). The chromosomal region 6q16.3-22.1 has shown linkage to bipolar affective disorder in single studies (Schultze TG et al, 2004) and meta-analysis (McQueen et al., 2005). One candidate gene located in that region is GRIK2, coding for an ionotropic glutamate receptor, GluR6. As a candidate gene for bipolar disorder, the exons and promoter region of GRIK2 were sequenced in 96 individuals from the NIMH genetics initiative collection diagnosed with bipolar disorder. 37 SNPs or rare variants were found in the GRIK2 gene in our study. Seventeen of the SNPs were known before and were reported in dbSNP. Twenty new SNPs or rare variants were found in our material. An additional 6 SNPs, previously reported to be located within the sequenced regions of the GRIK2 gene, were not found in our sample. Sequencing results may not reveal the presence of large heterozygous deletions of genomic sequence, since sequences from the other DNA strand do not show any abnormalities. However, such deletions lead to the presence of homozygous stretches of polymorphic markers within the deleted region. We have developed a software program that enables automatic search for long, homozygous stretches based on sequence data. The program takes into account local linkage disequilibrium (either based on the sequence data or public databases) and gives the user the option to specify several parameters such as definition of haplotype blocks and marker frequency cut-offs. We have identified two individuals with large stretches of homozygous markers, which are currently followed up by dense SNP genotyping.

Molecular determinants of Mammographic Density. *f. Odefrey[1], m. Southey[1], j. Stone[2], c. Apicella[2], s. Treloar[3], j. Hopper[2]* 1) department of pathology, university of Melbourne, Melbourne, VIC, Australia; 2) Centre for Molecular, Environmental, Genetic and Analytic Epidemiology, The University of Melbourne, VIC, Australia; 3) Centre for Military and Veterans Health, The University of Queensland, Herston Road, Herston QLD,Australia.

Mammograms have been used for decades for the detection of breast cancer (BC). This radiographic representation of the breast usually includes light/white areas (also called dense area, corresponding to the epithelial/stroma tissue) and non-dense (radiographically dark) area recognized as fat tissue (adipocytes) (Boyd et al. 1998). Mammographic Density (MD) has been shown to be associated with BC risk. A high percentage of MD (dense area as a proportion of total area of the breast) and a high absolute area MD are associated with an inter-quintile BC risk of about 4-fold (Boyd et al.,1998; Boyd et al.,2005).Twin studies have suggested that MD is highly heritable (Boyd et al., 2002). About 60% of the population variation in age-adjusted MD may be due to as yet unknown genetic variants. One approach to the investigation of the genetic determinants of MD has been to utilize the results of recent BC genome wide association studies (GWAS) that have identified common SNPs in or near several genes or loci (e.g. FGFR2, TNRC, MAP3K1, LSP1, H19 and 8q) that are associated with very small differences in BC risk (<10% per allele) and explain4% of the presumed population polygenic variance and a similarly small proportion of the population familial risk (Easton et al., 2007; Hunter et al., 2007). Given the relationship between BC and MD one or more of these common BC susceptibility variants could explain some of the variation in dense area, especially those suggesting a biological plausibility, such as FGFR2 which is involved in stroma development. We have genotyped the 7 SNPs that were the most strongly correlated with BC in the BCAC study (Easton et al., 2007). We used the Australian Twins and Sisters Mammographic Density Study (ATSMDS) which collected blood samples and mammograms for approximately 3000 Australian women (600 of whom are from monozygotic twin pairs). We will present the results of our investigation.

Search for genomic regions and genes within these regions possibly involved in MMR-related cancer. *H. Westers, P. van der Vlies, P. Jager, R. Sijmons, R. Hofstra* Genetics, University Medical Center Groningen, University of Groningen, P.O. box 30.001, 9700 RB, Groningen, the Netherlands.

Lynch syndrome is an autosomal dominantly inherited disorder that is the most common cause of hereditary colorectal cancer. It is well-established that germline mutations in the mismatch repair genes *MLH1*, *MSH2*, *MSH6*, and *PMS2* cause Lynch syndrome. Although microsatellite instability (MSI-H), a hallmark for mismatch repair deficiency, is a common feature in tumours from families clinically fulfilling the Amsterdam criteria for Lynch syndrome, mutations in the mismatch repair genes account for only 50 % of these Lynch syndrome families. In those families that do not fulfil the Amsterdam criteria but that do fulfil the less strict Bethesda criteria, only 20 % of the families have a pathogenic mutation. Our studies focus on the identification of genes that might explain the yet unsolved cases. Our database in which study data on Lynch Syndrome (suspected) families were collected consists of 132 HNPCC-suspected patients with one or more MSI-H tumours. 94 of these patients did not have a mutation in the three major MMR genes, *MLH1*, *MSH2*, and *MSH6*. Immunohistochemical (IHC) screening was performed and 32 of these patients had normal immunohistochemistry stainings for the proteins encoded by these genes. From these patients DNA was isolated from paraffin-embedded tumour tissue material (when available) for array CGH experiments. Hybridisations were performed on home-made BAC arrays and 105k Agilent arrays. The array CGH data that were obtained so far were analysed with NEXUS CGH software, which is a tool for the analysis of array CGH-based data from multi-array experiments supporting any platform. Some copy number changes were detected at chromosomes 3p (deletions), 8p (deletions), 8q (duplications), and 20q (duplications). Data on genes within these regions possibly involved in MMR-related cancer will be presented.

Complete sequencing of the coding region of the CFTR gene using the new generation GS-FLX sequencing technology. *H. Cuppens, L. Vliegen, J.-J. Cassiman* Center for Human Genetics, KULeuven, Leuven, Belgium.

The CFTR test is most probably the most common used genetic test in the Caucasian population. Given the heterogeneity of CFTR mutations, the sensitivity of the current mutation-specific CFTR tests mostly not exceeds 88-92%. A negative DNA test result does not guarantee absence of a mutation, which is especially of high relevance in the CFTR mutation screening programs that have been introduced. Only with sequencing, a 100% mutation detection rate is obtained in DNA fragments that are analyzed. This used to be too expensive. Sequencing costs, however, are going down through the introduction of next-generation sequencing technologies. Next-generation sequencing technology, such as picotiter pyrosequencing on a GS-FLX system, was initially developed for whole genome sequencing purposes. We have adopted this technology for complete sequence analysis of the CFTR coding region, and its exon/intron junctions. To this aim we have developed a robust multiplex amplification assay in which biotinylated amplicon-specific primers are locally restricted through streptavidin/biotin crosslinking. Indeed, 30 amplicons should be analyzed for the CFTR gene, and this can be only economically feasible if all amplicons are amplified in one, or a limited number, of PCR multiplex reaction(s). For a 50x coverage, only half a million nucleotides are needed for CFTR sequence analysis, i.e. 0.5% of the full capacity of the GS-FLX system. Therefore, 100-200 samples should be pooled in order to use the full capacity of the GS-FLX system. We therefore also developed an economically feasible universal sample tagging approach allowing the pooling of 100 samples with one set of 260 primers (60 CFTR amplicon-specific primers and 200 tagging primers). This compares to 6000 primers if amplicon-specific PCR primers are tagged as such. This assay now allows us sequencing of the CFTR gene in an economically feasible way, obtaining a mutation detection rate of 100%, with the potential of becoming the CFTR genetic test.

Oligonucleotide DNA microarray profiling of lung cancer revealed vasoactive peptide receptor 1 and osteopontin as potential biomarkers. *V. Mlakar[1], M. Strazisar[1], M. Sok[2], D. Glavac[1]* 1) Department of Molecular Genetics, Faculty of Medicine, Institute of Pathology, University of Ljubljana, Korytkova 2, 1000 Ljubljana, Slovenia; 2) University Medical Centre Ljubljana, Clinical Department for Thoracic Surgery, Zaloska cesta 2, 1000 Ljubljana, Slovenia.

Despite extensive research on lung adenocarcinoma (AD) using different DNA microarray platforms, recent evidence shows that the majority of genes involved in its development are still unknown, thus showing that the search for genes involved in lung carcinogenesis needs to continue in order to understand events leading to lung cancer development. The purpose of this study is to find novel gene(s) involved in the development of ADs using an oligonucleotide based DNA microarray platform with a selected set of genes. In order to analyse expression profile and expression of VIPR1 and SPP1 gene on 19 ADs and 5 squamous cell lung cancers (SCLC) microarrays and real time PCR were used. Moreover, real time PCR was used to assess gene copy number of the VIPR1 gene in all 24 samples. 31 up-regulated and 8 down-regulated genes were identified. Up-regulation of the SPP1 gene and down-regulation of the VIPR1 gene was confirmed using real time. We describe losses and gains of the VIPR1 gene in 6 and 3 ADs, respectively, and loss of the VIPR1 gene in 2 SCLCs. No correlation was observed between the histological status of ADs and the copy number of the VIPR1 gene. Samples with 1 and 3 copies of the VIPR1 gene had 2,2 times less and 15 times more VIPR1 mRNA than normal copy samples, respectively. No significant change was observed due to high variation of expression within 3 groups. We found reverse correlation between SPP1 and VIPR1 expression. Down-regulation of VIPR1 gene in ADs and SCLCs corroborates with its proposed tumour suppressor role. This observation is also supported by reverse association with SPP1. No association between changes in expression or copy number of VIPR1 gene with histological data shows that both, changes in expression and copy number, must occur early in AD development. The high variation in VIPR1 mRNA amounts also suggests that VIPR1 gene is extensively regulated.

Follow-up analysis of two independent genome-wide association studies of bipolar disorder provides evidence for *JAM3* as a susceptibility gene. *T. W. Muehleisen[1], J. Vollmer[1], S. Herms[1], M. Mattheisen[1], A. E. Baum[2], F. J. McMahon[2], C. C. Diaconu[3], M. Grigoroiu-Serbanescu[4], G. Babadjanova[5], V. Krasnov[6], R. Abou Jamra[7], J. Schumacher[2,7], R. Breuer[8], S. Witt[8], A. Georgi[8], T. G. Schulze[2,8], P. Propping[7], M. Rietschel[8], M. M. Nöthen[1,7], S. Cichon[1,7]* 1) Dept Genomics, Life & Brain Ctr, Univ Bonn, Bonn, Germany; 2) National Inst Mental Health, Bethesda, USA; 3) Stefan Nicolau Inst Virology, Bucharest, Romania; 4) Alexandru Obregia Psychiatric Hospital, Bucharest, Romania; 5) Russian State Medical Univ, Moscow, Russia; 6) Moscow Research Inst Psychiatry, Moscow, Russia; 7) Inst Hum Genet, Univ Bonn, Bonn, Germany; 8) Dept Genet Epidemiol in Psychiatry, Central Inst Mental Health, Mannheim, Germany.

The first two genome-wide association studies of bipolar disorder (BD) applied different approaches: Baum *et al*. (2007) analysed DNA pools from US-American and German samples on Illumina HH550 arrays and reported consistent findings between both samples; the Wellcome Trust Case Control Consortium (WTCCC, 2007) employed individual genotyping in a UK patient-control sample on Affymetrix 500K arrays. Here, we aimed to (1) identify genes that showed evidence for association in both studies, (2) individually genotype Affymetrix SNPs from these genes in our German sample, which was part of Baum *et al*., to make the results comparable, and (3) follow-up promising SNPs in independent BD samples. A gene-based comparison of both studies resulted in 9 genes with overlapping signals. From these genes, we selected 21 Affymetrix SNPs and individually genotyped them in the German sample (691 patients, 944 controls) and in an independent Russian sample (212 patients, 179 controls). We found that SNPs in *JAM3* (*junctional adhesion molecule 3*) were significantly associated with BD in both samples (best *P* value=1.95E-03). In summary, genetic variation in *JAM3* shows consistent association with BD in three independent samples (Baum et al, WTCCC, Russian) and can therefore be regarded as a very promising susceptibility gene for BD. To further strengthen our findings, we are currently testing *JAM3* SNPs in additional independent BD samples.

Integrating methods for detecting copy number variations in Han Chinese population. *T. P. Chuang[1], H. C. Yang[2], Y. J. Shih[1], J. Y. Wu[1], Y. T. Chen[1], L. H. Li[1]* 1) IBMS, Academia Sinica, 128 Sec. 2, Academia Rd. Nankang, Taipei 115, Taiwan; 2) ISS, Academia Sinica, 128 Sec. 2, Academia Rd. Nankang, Taipei 115, Taiwan.

After human genome was decoded, the idea that genomes between two individuals are similar has been challenged by more and more discoveries on genomic variations. Among all types of genomic variations, copy number variation (CNV) gets more and more attention in the post-genome era because of its potentiality in influencing gene expression, and thus susceptibility to diseases. Up to present, most reports of CNV study with large sample size are based on Caucasian and African populations. Since some genomic variations are ethnic specific and a large proportion of CNVs are at low frequency, it is necessary to identify CNVs in Han Chinese population with a large sample size. Here, we reported the construction of a fine-scale CNV map for Han Chinese residing in Taiwan. In this study, genomic DNA was isolated from EBV transformed B cell lines established from 390 unrelated healthy individuals. Subsequently, the DNA was genotyped by using Affymetrix GeneChip Human Mapping 500K Array Set. Intensity data was processed by using Partek Genomic Suite to detect copy number alterations. Moreover, regions with allelic imbalance (AI) resulted from long contiguous stretch of homozygosity (LCSH), deletion, or amplification were identified using Microarray Pooled DNA Analyzer (MPDA). A total of 2292 common autosomal CNV regions (CNVRs) were identified in the 390 individuals; among them, 865 were unique in Han Chinese. 257 out of the 865 (29.71%) CNVRs are 10kb, 497(57.46%) 10kb and 50kb, 97(11.21%) 50kb and 100kb, and 14(1.62%) 100kb. Collectively, 248 Han-Chinese unique CNVRs were deletion type, 463 were amplification type, and 154 possessed deletion and duplication at the same locus. Our preliminary results showed that 14 out of 16 tested regions were true positive while 2 were false positive. Intriguingly, in addition to autosomal CNVs, we observed whole chromosome gain or loss of chromosome X in some female subjects by copy number analysis. Whether this unexpected observation is biologically relevant or it is resulted from cell culture artifact will be further investigated.

Oligonucleotide array-based comparative genomic hybridization (CGH) analysis of MNS antigen genes of donors with rare phenotypes. *Y. Suto[1], H. Tsuneyama[2], M. Hirai[3], K. Ogasawara[1], M. Uchikawa[2], H. Okazaki[1], K. Tadokoro[1]*
1) Research and Development, Japanese Red Cross Central Blood Institute, Tokyo, Japan; 2) Japanese Red Cross Tokyo Metropolitan Blood Center, Tokyo, Japan; 3) IREIIMS, Tokyo Women's Medical University, Tokyo, Japan.

[Summary] This is the first report of a rare individual showing a heterozygous genotype of *En*/*MiV* analyzed by array-based comparative genomic hybridization (CGH) technique. [Purpose] The MNS blood group system consists of more than 40 distinct antigens. These antigens are carried on glycophorin A, glycophorin B or hybrid proteins that arise from rearrangements within the *GYP* gene family, *GYPA*, *GYPB* and *GYPE*. These genes are located on chromosome 4q31.2 and share over 95% sequence identity. In this study, we applied array-based CGH for donors with rare phenotypes of En^{a}FR-. [Methods] By serological screening of rare blood type donors at our center, a donor [A] showing M+N-S+s+Hil+, two donors [B] and [C] showing En(a-) and eight normal donors were selected. CGH analysis was performed using their lymphocyte DNA, 244K Human Genome CGH microarray and CGH Analytics (ver.9.5)(Agilent Technologies). Further analysis using a high-density custom CGH microarray covering the whole region of 5-*GYPA*-*GYPB*-*GYPE*-3 (Agilent Technologies) was performed. The result was confirmed by sequencing the genomic DNA and cDNA derived from reticulocyte mRNA of donors using appropriate primer sets. [Results and discussions] By high-resolution CGH analysis of the donor [A], a 95-kb deletion including 3-part of *GYPA* and the intergenic region between *GYPA* and *GYPB* was found. There was a partial loss of *GYPB*, whereas no loss was detected in *GYPE*. In the donors [B] and [C], a 104-kb deletion including intron 1 to exon 7 of *GYPA* and the intergenic region was detected. No distinct loss was detected in their *GYPB* and *GYPE* genes. Genotype of the donor [A] was suggested to be heterozygous for *En* and *MiV* (*GYPA/B* fusion gene for Hil+ phenotype). It was confirmed by DNA sequencing for the donor [A], where a normal *GYPB* and the fusion gene were identified. Breakpoints were suggested to lie within intron 3 of *GYPA* and its homologous region in intron 2 of *GYPB*.

Detection of genomic copy number changes in Estonian patients with mental retardation by SNP genotyping microarrays. *A. Kurg[1], K. Männik[1], O. Zilina[1], H. Puusepp[1,2], S. Parkel[1], P. Palta[1], A. Veidenberg[1], K. Õunap[2,3]* 1) Institute of Molecular and Cell Biology, University of Tartu, Tartu, Estonia; 2) Pediatrics Dept, University of Tartu, Tartu, Estonia; 3) Medical Genetics Centre, United Laboratories, Tartu University Hospital, Tartu, Estonia.

Mental retardation (MR) is a highly heterogeneous condition with a prevalence of ~1-3%. Despite extensive investigations the underlying reason remains unknown in about half of the cases. It was established that in up to 30% of the patients MR may be caused by changes in chromosomal structure. We started the first comprehensive study in Estonia in order to find out causative factors in families with idiopathic MR and help to shed light on molecular mechanisms underlying MR. To date, we collected more than 220 DNA samples from MR patients with normal karyotypes and their unaffected family members. Infinium-2 whole-genome genotyping assay with Human370CNV-Duo BeadChips (Illumina Inc.) was applied for detection of DNA copy number changes. Acquired data were analyzed using BeadStudio v3.1 (Illumina Inc) and QuantiSNP (Colella et al 2007) software. Relevant results were confirmed by RT-qPCR or FISH. To maintain the information an ad hoc database was developed allowing to integrate and manage the phenotype-related data and genomic profiles in order to distinguish genomic alterations with possible clinical significance. So far, first 115 individuals from 29 families were screened. About 20 DNA copy number changes per individual were detected, most of which are reported in the Database of Genomic Variants or present recurrently in our samples. In six families, possible disease-related imbalances were found: 3.9Mb del(15)(q13.1q13.2), 8.3Mb del(7)(q31.1q32.1), 3.9Mb del(2)(q37.1), 1.6Mb dup(X)(p22.31), 1.4Mb dup(7)(q11.23), 4.5Mb dup(11)(q24.3-q25) and 3.9Mb del(12)(p13.33-13.32) (the last two aberrations were found in the same patient). The detected rearrangements were not present in unaffected family members. Cases with similar phenotypes and aberration(s) in the overlapping regions are also reported in the DECIPHER database. Involvement of these aberrations in the etiology of MR is currently under investigation.

Rearrangement breakpoints of PARK2 and DMD are clustered and randomly distributed at the common fragile site, FRA6E and FRAXC, respectively. *J. Mitsui[1], Y. Takahashi[1], H. Tomiyama[2], S. Ishikawa[3], H. Yoshino[2], J. Goto[1], H. Aburatani[3], D. Smith[4], A. Brice[5], I. Nishino[6], N. Hattori[2], S. Tsuji[1]* 1) Dept of Neurology, Tokyo University, Japan; 2) Dept of Neurology, Juntendo University, Japan; 3) Research Center for Advanced Science and Technology, Tokyo University, Japan; 4) Dept of Laboratory Medicine and Pathology, Mayo Clinic Cancer Center, US; 5) Federal Institute for Neuroscience Research, Pitié-Salpêtrière Hospital, France; 6) National Center of Neurology and Psychiatry, Japan.

Background: Gross rearrangements in PARK2 and DMD have been frequently observed not only in germlines (patients with AR-JP and DMD/BMD) but also in cancer cell lines, but the genomic structure prone to rearrangements underling above observations have been poorly understood. Methods: We designed two high density microarrays to cover the entire PARK2 and DMD, which enable identification of rearrangement. The high resolution of the microarray enables design of PCR primers flanking the rearrangements. We conducted array CGH analyses on the 230 AR-JP cases and 120 cancer cell lines employing the microarrays for PARK2, and 144 DMD/BMD cases employing the microarrays for DMD. Results: We identified 162 breakpoints in 230 AR-JP patients and 32 breakpoints in 120 cancer cell lines. We also identified 135 breakpoints in 144 DMD/BMD patients. Interestingly most of the breakpoints are different, suggesting that these rearrangements have occurred independently. The rearrangement breakpoints of PARK2 are clustered and randomly distributed at the center of the common fragile site (CFS), FRA6E. In addition, the rearrangement breakpoints of DMD are also clustered and randomly distributed within the region of the FRAXC. Interpretations: Although several lines of evidences have demonstrated that CFS holds associations with somatic cell rearrangements in cancers, CFS has rarely drawn attention as genomic structures associated with germline rearrangements. Our observations, however, suggest that chromosomal instability associated with FRA6E and FRAXC play an important role in in vivo rearrangements not only in somatic cells but also in germinal cells.

Mutation analysis of exons coding transmembrane and intracellular part of Notch3 protein in patients with cerebral angiopathy different from CADASIL. *H. Vlaskova[1], M. Bouckova[1], L. Dvorakova[1], M. Hrebicek[1], R. Matej[2], M. Elleder[1]* 1) Institute of Inherited Metabolic Disorders, 1st Faculty of Medicine and University Hospital, Charles University, Prague, Czech Republic; 2) Czech National Reference Laboratory of Transmissible Encephalopathies and Creutzfeldt-Jakob disease, University Thomayer Hospital, Prague, Czech Republic.

CADASIL (Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy, OMIM 125310) is an inherited small vessel disease causing migraine, early strokes, cognitive impairment and premature death. The hereditary angiopathy is caused by mutations in NOTCH3 gene located at 19p13.2-p13.1. The mutations regularly result either in a gain or loss of cysteine residues in the EGF-like repeats in the extracellular amino-terminal region of Notch3 protein. Recently, a mutation was identified in the intracellular part in patient with small vessel disease (Fouillade et al., 2008). We have examined 20 probands with clinical suspicion of CADASIL and 18 family members by DNA analysis of the region encoding the extracellular part of Notch3 (23 exons). We have found the heterozygosity for cysteine changing mutations in six probands: p.C144F, p.R182C, p.Y189C, p.G296C (this mutation is novel), p.R332C and p.R421C. In other proband, there was a different missense mutation - p.V644D. This mutation was analysed by predictive programs as a non tolerated substitution. Inspection of other family members and segregation of p.V644D with phenotype would futher support our suggestion that the mutation is deleterious. Mutations in the extracellular part have not been identified in thirteen probands. In these patients we analysed mutations in the region encoding the transmembrane and intracellular part of Notch3 protein. We have found no pathologic variation as yet, but we identified 10 previously published SNPs. This early result supports hypothesis that pathogenic mutations in these parts of Notch3 protein are not common in patients with small vessel disease. Support: VZ MSM CR 0021620806, VZ MZ CR 64165.

Medical management patterns differ between physician specialties engaged in the care of patients with Hunter syndrome (MPS II). *G. Cohn* Shire HGT, Cambridge, MA.

Hunter syndrome is an X-linked inborn error of glycosaminoglycan metabolism caused by a deficiency in the activity of the lysosomal enzyme, iduronate-2-sulfatase. Multiple organs and systems are adversely affected in MPS II, and thus the management and treatment of patients is often provided by multiple specialists (eg, pediatricians, medical geneticists, pulmonologists). This study was conducted to determine whether medical management differs between various specialists engaged in the management of individuals affected with Hunter syndrome. Methods:The Awareness, Trial and Usage Study was a market research survey commissioned by Shire Human Genetic Therapies, Inc. to develop a greater understanding of the management and treatment of Hunter syndrome, including the use of enzyme replacement therapy (ERT). Physicians engaged in the medical management of Hunter syndrome in the US were surveyed regarding their practice specialty, the number of MPS II patients managed, whether ERT had been discussed or recommended, and what surveillance strategies they used for patients receiving ERT. Results: Seventy-five physicians managing a total of 244 MPS II patients responded. Among those surveyed, physicians identified themselves as pediatricians (P) (47%), medical geneticists (MG) (44%), or other (O) (9%). The percentage of physicians that did not discus the option of ERT varied by specialty (P 37%, O 14%, MG 9%, ^{2} =7.93, P = 0.02). Furthermore, a significant difference in the reported use of abdominal measurement (A) and echocardiography (E) for the surveillance of patients prescribed ERT was observed between MG and P (A: MG 75%, P 33%, ^{2}=5.43, P=0.02; E: MG 81%, P 46%, ^{2}=4.04, P=0.04). Conclusions: The medical management of Hunter syndrome appears to differ between various medical specialties, as do surveillance strategies for patients receiving ERT. Where appropriate, consensus should be sought on management strategies for MPS II patients. Practice guidelines developed by the various medical specialties involved in the management of MPS II may be helpful in encouraging greater uniformity and consistency in the care of individuals with Hunter syndrome.

New target genes for microsatellite instable endometrial tumors. *A. M. Monteiro Ferreira[1,5], F. Gerbens[1], H. Westers[1], K. Kooi[1], K. Bos[1], C. Esendam[1], T. van der Sluis[2], M. Zazula[4], J. Stachura[4], A. G. van der Zee[3], R. Seruca[5], H. Hollema[2], R. M. W. Hofstra[1]* 1) Department of Genetics, University Medical Center Groningen, Netherlands; 2) Department of Pathology, University Medical Center Groningen, Netherlands; 3) Department of Gynecology, University Medical Center Groningen, Netherlands; 4) Department of Patomorfology, Medical College, Jagiellonian University, Poland; 5) Institute of Molecular Pathology and Immunology of the University of Porto, Portugal.

Endometrial carcinoma (EC) is the most common extra colonic cancer in Lynch syndrome. This syndrome is characterized by functional inactivation of mismatch repair (MMR) genes. MMR deficiency leads to an accumulation of mutations at microsatellites, leading to a phenotype known as microsatellite instability (MSI-H). Genes containing microsatellites are therefore frequent targets of mutations in tumors with MMR deficiency. Previous studies show that the profile of affected target genes differs between MSI-H EC and colorectal tumors (CRC), and that important target genes remain to be found. The aim of this study was to identify new target genes for MSI-H EC. 20K expression microarrays were used to select a total of 2338 genes highly expressed in normal endometrial tissue, which were then screened for repeats in their coding sequence. 574 repeats (in 432 genes) were sequenced in 40 MSI-H EC samples. Mutations were found in 47 genes and mutation frequencies equal or higher than 15% were found in 7 of those genes. Surprisingly, none of these has been previously reported either in MSI-H EC or CRC. Our list includes genes encoding proteins with various functions in the cell, such as a docking protein, methylation-related proteins and tyrosine-kinases. Interestingly, the second gene on the top list, with a 33% mutation frequency is a gene encoding a known co-repressor protein of the estrogen-receptor pathway. Functional studies are being done at our group and have to prove how these genes are involved in EC development. However, we believe that our results give new insights in EC development and might prove helpful in developing (new) EC therapeutics.

Identification of a novel PRPF31 mutation in a multiplex family segregating retinitis pigmentosa (RP) with highly incomplete penetrance. *J.-M. Rozet[1], N. Delphin[1], A. Munnich[1], J.-L. Dufier[2], O. Roche[2], J. Kaplan[1]* 1) Genetics, INSERM U781 & Université Descartes, Paris, France; 2) Dpt Ophthalmology, Hôpital des Enfants Malades, Paris, France.

Retinitis pigmentosa (RP) are the most common inherited retinal dystrophies. They are widely genetically and clinically heterogeneous. We report here the genetic study of a 13 year-old girl presenting with night blindness and an annular scotoma. While her visual acuity was strictly normal, these symptoms suggested the diagnosis of RP. Her maternal first-cousin, a 21 year-old male, was reported to be affected with RP since the age of nine. Besides, her maternal uncle and a first cousin of the maternal grand-mother were known to be affected with RP. The aim of the present study was to identify the disease gene in this family segregating RP with uncertain mode of inheritance. We first considered X-linked transmission but no linkage was found at the RP2, RP3 and RP24 loci, respectively. Besides, the mother of the proband and the mother of her first-cousin did not complain of visual dysfunction and had normal ERGs while that of their respective child were close to extinction. The lack of visual dysfunction, the absence of refraction errors and the normality of ERG recordings in the mothers prompted us to exclude the X-linked transmission. Subsequently, we considered the autosomal dominant transmission of the disease with incomplete penetrance and the involvement of the PRPF31 gene. Indirect studies at the RP11 locus were compatible with this hypothesis and the direct sequencing of the gene revealed the segregation of a novel missense mutation in all affected and some unaffected members of the family. Interestingly, the segregation of the second PRPF31 allele in individuals harbouring the mutation suggests the existence of a PRPF31 trans-element acting as a modifier of the phenotype. This hypothesis will be studied by examining the expression of the second PRPF31 allele.

Preliminary Results of a Phase 2 Clinical Trial of Genz-112638 in Patients with Type 1 Gaucher Disease. *N. Watman[1], E. Lukina[2], E. A. Arreguin[3], M. Banikazemi[4], M. Iastrebner[5], H. Rosenbaum[6], A. Zimran[7], F. O'Brien[8], S. E. Smith[8], A. C. Puga[8], J. Peterschmitt[8]* 1) Hospital Ramos Mejia, Buenos Aires, Urquiza, Argentina; 2) Hematology Research Center of Russian Academy of Medical Sciences, Moscow, Russia; 3) 2Hematology Research Center of Russian Academy of Medical Sciences, Moscow, Russia; 4) NYU, New York, USA; 5) Instituto Argentino de Diagnostico y Tratamiento, Buenos Aires, Argentina; 6) Rambam Medical Center, Haifa, Israel; 7) Shaare Zedek Medical Center, Jerusalem, Israel; 8) Genzyme Corporation, Cambridge, USA.

Introduction: Genz-112638, an investigational compound, is a novel oral small molecule inhibitor of glucosylceramide synthase being developed for the treatment of Gaucher disease type 1 (GD1). Objective: To assess the efficacy, safety, and pharmacokinetics of Genz-112638 in patients with GD1. Methods: An ongoing open-label Phase 2 clinical trial of Genz-112638 (50 or 100mg bid orally) enrolled patients with GD1 in Israel, North America, Russia, and South America. The main efficacy endpoints of the study were changes in hemoglobin level, platelet count, and spleen volume after 52 weeks. An extension study will follow. Results: Data were available for up to 21 and 13 patients receiving Genz-112638 at 26 and 52 weeks, respectively. All 9 males and 12 females (age range: 18-55y) were Caucasian. Four were of Ashkenazi Jewish and 1 was of Hispanic descent. At 26 and 52 weeks mean hemoglobin increased by 0.9g/dL and 1.3g/dL, respectively; mean platelet count increased by 18% and 34%; mean spleen volume decreased by 27% and 40%; mean liver volume decreased by 7% and 14%; mean chitotriosidase activity decreased by 30% and 50%. Plasma glucosylceramide levels normalized in all patients. Seven related adverse events were reported in 6 patients and all were mild and transient in nature. Conclusions: Initial observations suggest that Genz-112638 may represent a safe, effective, and convenient oral therapy for patients with GD1. Clinical development of Genz-112638 will proceed in ongoing and additional clinical trials.

Mavlonic Aciduria in a Child Featuring Hepatic Fibrosis and Novel Mevalonate Kinase Mutations. *Y. Anikster, N. Goldstein, M. Harel-Meir* Metabolic Disease Unit, Safra Children's Hosp, Tel-Hashomer, Israel.

Mevalonic aciduria (MVA, OMIM# 610377) is a rare inborn error of isoprene biosynthesis featuring recurrent attacks of fever, developmental delay, ataxia, dysmorphic features, failure to thrive, cataracts, and retinal dystrophy. Mutations in the mevalonate kinase (*MVK*) gene responsible for MVA, also account for Hyper Immunoglobulin D syndrome (HIDS), an autosomal recessive periodic fever syndrome. Most cases of HIDS and MVA have been described in patients of western European ancestry, mostly Dutch or French. The following report details a Palestinian patient diagnosed with MVA. This patient presented with some of the typical signs and symptoms of MVA including hepatosplenomegaly, recurrent fevers, and a developmental delay, as well as with scantly described phenotypic features such as hepatic fibrosis. Sharing clinical features of both MVA and HIDS, this patient emphasizes the phenotypic continuum of these two entities. Molecular analysis of this patient's DNA revealed a unique newly reported genotype - compound heterozigousity to V8F (t25a), and F38I (t112a). Although *MVK* gene mutations have been previously described in a Palestinian HIDS patient, this to our knowledge is the first report of MVA in a patient of Palestinian descent, and the first MVA patient to be described in Israel.This case emphasizes that MVA must be included in the differential diagnosis of hepatic fibrosis.

Characterizing genetic variants predisposing to MS in a Finnish sub-isolate. *V. Leppa[1], E. Jakkula[1,2], J. Saarela[1], S. Kallio[1], P. Tienari[3], K. Koivisto[4], S. Purcell[2,5], A. Palotie[1,2,6], M. J. Daly[2,5], L. Peltonen[1,2,6]* 1) Institute of Molecular Medicine (FIMM), National Public Health Institute and Univ of Helsinki, Helsinki, Finland; 2) The Broad Institute of MIT and Harvard, Cambridge, MA, USA; 3) Dept. of Neurology, Helsinki Univ. Central Hospital, Helsinki, Finland; 4) Central Hospital of Seinäjoki, Seinäjoki, Finland; 5) Center for Human Genetic Research, Massachusetts General Hospital, Boston, MA, USA; 6) Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK.

Prevalence of multiple sclerosis (MS) in the Southern Ostrobotnia region of Finland with a limited number of founders is two fold compared to the rest of Finland. We used genealogical information reaching up to 15 generations back to construct two regional megapedigrees. We hypothesize that one or more relatively penetrant variants predisposing to MS may be regionally enriched and shared haplotype analysis can be used to identify MS loci using a genome wide, high density SNP screen. 72 MS cases and 68 controls from Southern Ostrobotnia were genotyped using the Illumina 317K HumanHap panel. A five SNP sliding window haplotype analysis was used and 46 regions were found to be associated with p-value 10^{-4}. These haplotypes were then extended to putative recombination hotspots and their frequencies estimated in two additional control groups (regional and general population, n= 175/466). Twenty-one of these regions were found to have at least 10% haplotype frequency difference between the MS cases and all three control groups and were further analyzed in an independent sample set from the high-risk region (n= 125/MS and 360/controls). Six of the regions (1p32, 1q25.3, 3p14.2, 5p13.1, 6p21, 17q11.2) showed association ($p=10^{-2}$-10^{-6}) in the independent replication set, including the HLA-DRB1. We are currently evaluating the role of the identified risk alleles in MS susceptibility of the general population in 700 MS cases and 700 controls. This approach offers a complementary strategy to large scale GWAs and should provide insight to putatively more penetrant risk alleles enriched in special populations.

Role of DNA copy number variation in susceptibility to childhood cancer. *A. Shlien[1,2], U. Tabori[1,3,5], C. R. Marshall[1,4], M. Pienkowska[1], L. Feuk[1,4], A. Novokmet[1,5], S. Nanda[5], H. Druker[5], S. W. Scherer[1,4], D. Malkin[1,2,3,5]* 1) Genetics & Genome Biol; 2) Medical Biophysics; 3) Pediatrics; 4) Centre for Applied Genomics; 5) Hematology/Oncology, Hospital for Sick Children, Canada.

DNA copy number variations (CNVs) are a significant form of inter-individual genetic variation, affecting more than 10% of the human genome. The size and plasticity of CNV regions makes them particularly intriguing to the study of cancer, yet their potential as genetic risk factors to cancer predisposition has not been explored. Li-Fraumeni syndrome (LFS) is an autosomal dominantly inherited disorder associated with germline mutations of TP53 which is characterized by an increased risk of early-onset breast cancer, sarcomas, brain tumors and other neoplasms. LFS is clinically and genetically heterogeneous and TP53 mutational status alone does not explain the variable phenotype in these families. As part of a wider study of CNVs and cancer, we conducted a genome-wide profile of germline CNVs in LFS families. We examined genomic DNA from a large healthy population (n=770) and an LFS cohort using high-density arrays and show that the number of CNVs per genome is well-conserved in the healthy population, but noticeably increased in cancer-prone individuals. This represents a significant increase in CNVs among carriers of germline TP53 mutations with a familial cancer history ($p=0.01$). Further, our data suggests an association between the number of CNVs and the severity of the phenotype in these cancer-prone families. Refined mapping of these regions in pediatric cancer patient blood and tumor have led to the observation that CNVs are fertile ground on which larger somatic chromosomal deletions and duplications can develop in cancer cells. We continue to examine the mechanisms by which specific germline CNVs can inform the more dramatic chromosomal changes of TP53-related tumors. Our results suggest that examining CNVs in families predisposed to cancer may identify individuals with an abnormally high number of these events and provide insight into the earliest genomic alterations associated with the human carcinogenic process.

Cleidocranial Dysplasia RUNX2 R225Q Mutation Alters Dental Cell Gene Profile. *J. Dong[1], I. Gay[1], S. Chen[2], M. MacDougall[1]* 1) University of Alabama at Birmingham, School of Dentistry, Institute of Oral Health Research, Birmingham, AL; 2) University of Texas Health Science Center at San Antonio, Dental School, San Antonio, Texas.

Cleidocranial Dysplasia (CCD, MIM 119600) is an autosomal dominant condition that affects skeletal and tooth development characterized by clavicular hypoplasia, short stature, supernumerary teeth, and delayed tooth eruption. Mutations in the transcription factor RUNX2 (also known as CBFA1) have been shown to cause CCD. In this study we analyzed the potential consequences of a recurrent RUNX2 R225Q mutation in dental cells obtained from a CCD family seen in our dental clinic. Supernumerary teeth extracted for clinical reasons were used to establish primary periodontal ligament (PDLR225Q) cell cultures for analysis of RUNX2 cellular localization and gene expression profiles. Extracted CCD supernumerary teeth were obtained, PDL cells were isolated and primary cells were established. PDLR225Q cells as well as age/gender matched controls (PDLR225) were grown for phenotypic analysis using immunochemistry and gene microarray. Total RNA was hybridized to a human U133 GeneChip (Affymetrix) and altered genes expression levels (>2 fold) were confirmed by qRT-PCR. Our results indicate that the PDLR225Q cells showed decreased proliferation rates and accumulated RUNX2 protein in the cytoplasm. Microarray analysis revealed disregulation of 38% of the genes tested: 5520 (14%) up-regulated and 9462 (24%) down-regulated. The genes confirmed by qRT-PCR included members of the BMP and FGF signaling pathways such as dentin sialophosphoprotein, osteonectin and MMP-20. Selected target genes were shown to have potential RUNX2 binding sites in their 5 promoter sequences. These results indicate that the RUNX2 R225Q interferes with nuclear transport resulting in a haploinsufficiency that leads to disregulation of a large cadre of genes critical for normal tooth development. Supported by UAB SOD IOHR and Deans Faculty Award.

High-Resolution Mapping and Analysis of Copy Number Variations in the Human Genome. *P. S. White[1,2], X. Gai[1], T. H. Shaikh[1,2], J. C. Perin[1], J. T. Glessner[1], H. M. Xie[1], T. Casalunovo[1], M. D'arcy[1], E. C. Frackleton[1], C. E. Kim[1], K. Murphy[1], R. O'Hara[1], R. Grundmeier[1,2], M. Imielinski[1], S. Ostapenko[1], R. M. Chiavacci[1], E. F. Rappaport[1], S. F. A. Grant[1,2], H. Hakonarson[1,2]* 1) Joseph Stokes, Jr. Research Institute, Children's Hospital of Philadelphia, Philadelphia, PA, 19104, USA; 2) Department of Pediatrics, University of Pennsylvania, Philadelphia, PA, 19104, USA.

There is growing evidence that copy number variation (CNV) in the human genome significantly influences human diversity and predisposition to disease. However, the frequency and distribution of copy number variations (CNVs) in the human genome remains to be characterized in large sample sets of healthy subjects. We catalogued and characterized copy number variations (CNVs) in 2,026 disease-free individuals. A total of 54,462 CNVs were detected, of which 77.8% were identified in multiple unrelated individuals. These non-unique CNVs mapped to 3,272 distinct regions of genomic variation spanning 5.9% of the genome; 51.5% of these were previously unreported, and >85% are rare. Certain CNVs demonstrated significant frequency differences between Caucasians and African Americans, indicating the presence of ethnic-specific CNV signatures. Our CNV set distribution is positively correlated with regions of segmental duplication, long interspersed repeats (LINEs), and recombination hot spots. CNVs were found to be negatively correlated with gene density in general, but the subset of genes previously implicated in human disease were found to be more enriched within the CNV regions, suggesting that CNVs may influence disease susceptibility through natural selection. Certain gene functional categories were also found to be preferentially associated with CNVs. We demonstrated the clinical utility of this CNV set in distinguishing a pathogenic CNV from normal variants in a patient with multiple congenital anomalies. Together, this analysis and annotation provides a timely resource to assist with the assessment of CNVs in the contexts of human variation, disease susceptibility, and clinical molecular diagnostics.

Identification of Novel Benign Juvenile Epilepsy Gene in Dogs. *E. H. Seppala[1,2], T. Jokinen[1,2], A.-K. Anttonen[1,2,3], M. Kousi[1,2,3], S. Cizinauskas[4], E. Karlsson[5], B. Minassian[6], M. Perloski[5], M. Webster[7], T. Leeb[8], K. Lindblad-Toh[5,7], H. Kalimo[9], A.-E. Lehesjoki[1,2,3], H. Lohi[1,2]* 1) Dept of Medical Genetics and Dept of Basic Veterinary Sciences, Univ of Helsinki, Finland; 2) Folkhälsan Institute of Genetics, Finland; 3) Neuroscience Center, Univ of Helsinki, Finland; 4) Referral Animal Neurology Hospital Aisti, Vantaa, Finland; 5) Broad Institute of Harvard and MIT, Cambridge, USA; 6) Hospital for Sick Children, Toronto, Canada; 7) Dept of Medical Biochemistry and Microbiology, Uppsala Univ, Sweden; 8) Inst of Genetics, Vetsuisse Faculty, Univ of Bern, Switzerland; 9) Dept of Pathology, Univ of Helsinki, Finland.

Childhood epilepsies with benign outcomes are well-known and several genes have been identified. Epilepsies are common in dogs too but only five genes have been discovered in progressive myoclonic epilepsies. We have recently identified a new epilepsy syndrome in Lagotto Romagnolo (LR) breed. LRs manifest benign juvenile epilepsy with simple and complex focal seizures appearing in puppies at the age of five to nine weeks. Seizures resolve spontaneously at the age of eight to thirteen weeks. EEG activity is present in most affected puppies and some dogs present neurological signs including generalized ataxia and hypermetria. MRI, EMG and brainstem auditory-evoked potentials appear normal. Histopathology revealed cerebellar inclusions in Purkinje cells. To map the epilepsy gene we performed genome-wide association analysis using Affymetrix 27K SNP array in 11 sib pairs. We recognized a single 2.7Mb critical region in the chromosome 3 containing ten genes. Evaluation of the genes revealed an obvious candidate gene for sequencing. We identified a homozygous nonsense mutation in affected dogs resulting in the truncation of the protein. The identified gene has not been associated with epilepsy before. The function of the gene is not known and our ongoing studies aim to discover its role in epileptogenesis. We are also sequencing the gene in human epilepsy patients. Identification of the LR gene establishes the breed as a model for childhood epilepsies and will improve our understanding of juvenile epilepsies both in human and dogs.

Identification of a new mutation in FGD4. Discussion of the phenotypic heterogeneity in Charcot-Marie-Tooth disease 4H: implications for molecular diagnosis. *C. BAUDOT[1], Y. POITELON[1], T. HAMADOUCHE[2], I. BOCCACCIO[1], A. MEGARBANE[3], N. LEVY[1,4], V. DELAGUE[1]* 1) UMR_S 910, INSERM, Faculte de Médecine de la Timone, Université de la Méditerranée, Marseille, France; 2) Laboratoire de Biologie Moléculaire, Institut Pasteur, Algers, Algeria; 3) Unité de Génétique Médicale, Université Saint-Joseph, Beirut, Lebanon; 4) AP-HM, Département de Génétique Médicale, Hôpital d'enfants Timone, Marseille, France.

Charcot-Marie-Tooth (CMT) disorders are a clinically and genetically heterogeneous group of hereditary neuropathies characterized by chronic distal weakness and sensory loss. We recently identified FGD4, encoding the Rho GDP/GTP Exchange Factor (RhoGEF) FRABIN, is the gene responsible for CMT4H, a rare autosomal recessive demyelinating CMT subtype, that we previously mapped at chromosome 12p11.21-q13.11, in two consanguineous families of Mediterranean origin (De Sandre-Giovannoli et al.2005). We identified two mutations at the same codon in exon 7 of the gene: the c.893T>G transversion and the c.893T>C transition in a Lebanese and an Algerian family respectively (Delague et al 2005). Whereas the mutation in the Algerian is a missense mutation (p.Met298Thr), the c.893T>G transversion proved to be a splicing mutation leading to a premature STOP codon (p.Met298fsX8). Subsenquently, Stendel et al.(2007) identified three other nonsense (c.670C>T, p.Arg224X and c.1756G>T, p.Gly586X)) or frameshift (c.1627_1628delGA, p.E543fsX5) mutations in exons 5, 15 and 13 respectively. In this study, we present the results of the screening of FGD4 in a cohort of 96 patients affected with CMT, and for whom no mutations have been identified to date in any of the known CMT genes. We describe the identification of a new missense mutation in FGD4 in one Lebanese girl and discuss the phenotypic heterogeneity among published patients with mutations in this gene. Identification of a new mutation in FGD4/FRABIN points to a major role of FRABIN in the pathogenesis of CMT disease. Our results also clearly suggest that the screening of FGD4 should be performed not only in early-onset, slow progressive forms of CMT, but also in later-onset, less severe forms.

An overall very mild phenotype associated with the CFTR mutation R117H, irrespective of the second mutation.

C. Thauvin-Robinet[1,3], F. Huet[2,3], M. P. Audrezet[4], C. Férec[4], M. Des Georges[4], M. Claustres[4], G. Lalau[4], F. Cabet[4], E. Bieth[4], G. Bellis[5], D. Hubert[2], R. Nové-Josserand[2], A. Munck[2,3], G. Bellon[2], C. Binquet[6], L. Faivre[1], M. Roussey[2,3], E. Girodon[4,7], R117H Collaborativ Group 1) Genetics Centre, Dijon,France; 2) CF Care Centres, France; 3) AFDPHE,Paris,France; 4) CF laboratory Network,France; 5) INED,Paris,France; 6) CIC-EC,Dijon,France; 7) Molecular Genetics Laboratory,Créteil,France.

Over 1500 CFTR sequence variations have been identified in cystic fibrosis (CF) and CFTR-related disorders (CFTR-RD), such as congenital bilateral absence of the vas deferens (CBAVD). They can be of severe, mild, or neutral clinical effect. R117H mutation, initially considered as a mild CF-causing mutation with pancreatic sufficiency and rare early pulmonary symptoms, was found predominantly associated with pure CBAVD, and incidentally observed in asymptomatic individuals. Out of a large French collaborative study collecting 278 patients until 2007 with two CFTR mutations including at least one R117H, we explored data from 179 patients recruited on the basis of symptoms or a positive family history. Median age at last follow-up was 31.5 years (IQR [27.3-35.7]). Clinical data available in 165 patients revealed 49% pure CBAVD, 40% other CFTR-RD, 8% totally asymptomatic individuals, and 3% with a severe pulmonary disease (FEV1 <60%) of late onset. 42 different mutations were identified: 95% of severe (F508del: 68%), 3% of mild and 2% of unknown effects. The IVS8 T5 variant was found in only 5 patients. Phenotype-genotype correlation studies showed no significant clinical difference between patients, according to the mutation class of the 2nd allele. This study confirmed the overall mild phenotype associated with compound heterozygosity for R117H and a severe mutation, irrespective of the mutation class. Even if the numbers were too small to obtain a significant difference, we observed that the 8 patients with mild mutation of the 2nd mutation had CBAVD, and only 3 also presented mild asthma. The very low frequency of mild mutations on the 2nd allele (3%) let to suggest that the clinical penetrance of genotypes combining R117H and a mild mutation is very low.

Testing haplotype association using Bayesian structural equation models. *T. Price* MRC SGDP Centre, Institute of Psychiatry, London, United Kingdom.

I propose a test of haplotype association embedded in a Bayesian structural equation model, a versatile method that allows one to test complex causal hypotheses involving both latent and observed variables. In the simplest case of a single response variable, the technique is equivalent to testing haplotype association within a generalized linear model using the prospective likelihood. Using a demonstration dataset, I apply this method to simultaneously test hypotheses about haplotype-environment association and haplotype-environment interaction. The potential applications for this technique are vary varied indeed, but include testing the causal influence of haplotypes on (a) multiple interrelated phenotypes; (b) latent factors loading on multiple observations, including growth curves or latent trajectories abstracted from longitudinal measurements; (c) the utility of treatment in a Bayesian decision-theoretic model incorporating personalized predictors of cost and efficacy.

Neuropathology of Chromosome 15 Duplication and Autism. *W. Brown, T. Wisniewski, i. Cohen, E. London, M. Flory, H. Imaki, I. Kuchna, J. Wegiel, S. Ma, K. Nowicki, K. Wang, J. Wegiel* NYS Inst Basic Research, Staten Island, NY.

Background: Duplications of the proximal long arm of chromosome 15 cause mental retardation, seizures, autistic features, functional deterioration with age and increased risk of sudden death. The pattern of neuropathological changes caused by this genetic defect is not known. Objective: Detection of (a) developmental changes contributing to mental retardation, (b) markers of neuronal degeneration contributing to clinical deterioration, and (c) pathology contributing to sudden death. Methods: Brains of four subjects (11, 15, 20, 25 year old) with chromosome 15 duplication, and four age matched control subjects were examined in the light and electron microscope. Results: The most consistent finding is a reduced size of the brain, volume of neurons and neuronal nuclei in the amygdala, accumbens, entorhinal cortex and Purkinje cells, indicating a delay in neuronal development. Microdysgenesis in the hippocampus represented by hyperconvolution and duplication of the granule cell layer in the dentate gyrus observed in two subjects may contribute to seizures and sudden death. Enhanced accumulation of amyloid beta protein in phagosomes/dense bodies in all four subjects is a marker of modified APP processing and amyloid beta trafficking and deposition. Amyloid beta positive inclusions in the dendrites in the CA1 sector are another marker of neuronal deterioration. Osmophilic inclusions in the mitochondrial matrix might be an indicative of mitochondrial degeneration. Chaslin's gliosis observed in one subject reflects epilepsy-related brain damage. Conclusion: In subjects with chromosome 15 duplications reduced size of the brain and delayed growth of neurons appears to be the main contributor to developmental deficits, whereas behavioral worsening could be the result of early neurodegeneration. Sponsor: Autism Speaks and the NYSOMRDD. The Harvard Brain Tissue Resource Center (R24-MH 068855), and Brain and Tissue Bank at the University of Maryland, Baltimore provided tissue. Autism Tissue Program coordinated tissue acquisition.

Genetic testing for epidermolysis bullosa in a commercial setting. *E. Pfendner, S. Bale, J. Compton* GeneDx Inc, Gaithersburg, MD.

Epidermolysis Bullosa (EB) is an inherited muco-cutaneous disorder characterized by skin fragility due to relatively minor trauma and exhibiting a broad spectrum of severity. Ten genes are known to be involved in the three major and two minor EB subtypes. Since testing for the Dystrophic (DEB) and Junctional (JEB) forms of EB was instituted at GeneDx in 2005, 56 samples have been studied for mutations in the COL7A1 gene mutated DEB, 36 samples for mutations in the 4 genes involved in JEB; (LAMB3, LAMC2, LAMA3 and COL17A1), and 9 samples in the ITGB4 gene involved in EB with pyloric atresia (EB-PA). Full coding region sequencing was employed to examine the candidate genes for mutations and resulted in a detection rate of 99% of COL7A1 mutations (16 autosomal dominant cases with 16 of 16 alleles detected and 40 autosomal recessive cases with 79 of 80 alleles detected) and 94% of JEB mutations (64 mutations from 68 alleles). Of the 34 JEB samples submitted, 20 harbored mutations in the LAMB3 gene, 7 in the LAMA3 gene, 4 in the LAMC2 gene and 3 in the COL17A1 gene. Of the 20 samples harboring LAMB3 mutations 10 carried one of the common mutations (R42X, R635X, 957ins77) on both alleles (50%) and 5 (25%) carried one of the common mutations on one allele. Nine samples were submitted with a diagnosis of EB-PA for ITGB4 sequencing and mutations were identified on one or both alleles in 7 cases. Detection rate for ITGB4 was 79% (11 of 14 alleles) with the two remaining cases presumably harboring mutations in one of the two EB-PA genes that were not studied (ITGA6 and PLEC1). Compared with results from the academic laboratory offering testing on a research basis during an equivalent time period, sample submission to GeneDx was 18% lower (101 vs 123 samples submitted) while detection rate overall was higher (99% vs 77% for DEB, 94% vs 76% for JEB, 79% vs 86% for ITGB4). The cost of testing is presumably responsible for lower sample volume while methodology (sequencing vs dHPLC screening followed by sequencing) was responsible for the higher detection rates. Novel mutations were identified in each of the genes studied.

GWA data of a founder population highlights substructure and multiple bottlenecks reflected by wide LD intervals and homozygosity regions. *E. Jakkula[1,2,9], K. Rehnström[1], T. Varilo[1], O. P. H. Pietiläinen[1], T. Paunio[1,3], N. L. Pedersen[4], N. Freimer[5], S. Ripatti[1,4], S. Purcell[2,6], A. Collins[7], M. J. Daly[2,6], A. Palotie[2,8,9], L. Peltonen[1,2,8]* 1) NPHI and Institute for Molecular Medicine (FIMM), Helsinki, Finland; 2) The Broad Institute of Harvard and MIT, Cambridge, MA, USA; 3) Helsinki Univ Central Hospital, Helsinki, Finland; 4) Karolinska Institutet, Stockholm, Sweden; 5) Univ of California, Los Angeles, California, USA; 6) Massachusetts General Hospital, Boston, USA; 7) Univ of Southampton, Southampton, UK; 8) Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK; 9) FIMM, Univ of Helsinki, Helsinki, Finland.

The genome-wide SNP datasets enable detailed association studies, but also offer new insight into population genetics. Here we present an example of a founder population by scrutinizing 11 geographically and historically distinct Finnish subpopulations representing different eras in the inhabitation.

Multidimensional scaling of pairwise identity-by state (IBS) sharing matrix was performed to delineate the population structure. The two primary dimensions corresponded with the east-west and north-south directions closely reflecting the geographical distribution of parents birthplaces. The youngest subisolates in northeastern Finland showed highest within-group IBS similarity, widest LD intervals and highest number of extended regions of homozygosity. These parameters were slightly higher in early settlement southwestern regions compared to CEU and Swedish samples. Tests of IBS similarity and genomic inflation factors revealed significant differences compared to general Finnish population of the capital.

The patterns identified correlate remarkably well with population sub-divisions based on extensive historical and linguistic information, and clarify the impact of consecutive bottlenecks and founder effects on the current population. Further, this data provides useful information for future studies aiming at identification of rare variants in common diseases, potentially enriched in subpopulations with fewer founders.

(dCA)10 and (dTG)10 oligonucleotide invasion into homologous internal region of DNA duplexes containing CA/TG repeats. *N. V. Ryadninskaya, V. K. Gasanova, A. A. Belyakova, G. A. Belitsky, M. G. Yakubovskaya* Institute of Carcinogenesis, Blokhin Cancer Research Center RAMS, Moscow, Russian Federation.

CA/TG microsatellites are well known to be the hot spots of genetic recombination. One of the reasons for this phenomenon may be formation of nonB DNA structures by CA/TG repeats because of intra- and intermolecular DNA-DNA interactions. Previously we have shown that oligonucleotide of random sequence could invade into homologous duplex end via consecutive strand displacement mechanism, while oligonucleotide invasion into the internal homologous region of the duplex have never been observed. In this work we studied oligonucleotide invasion into different types of duplexes containing (CA/TG)31-repeats: 1129 bp PCR product, 835 bp restricted fragment of plasmid DNA and 2384 bp circular plasmid. Purified PCR products and restricted fragments were incubated with radioactively labeled 20-mer oligonucleotides homologous either to the duplex end sequence or the internal CA-repeat region using molar ratio duplex/oligonucleotide equal to 1/50. After mixture incubation the probes were loaded on agarose gel followed by electrophoresis and gel autoradiography. Formation of invasion complexes was visualized by appearance of radioactivity in the duplex bands. For the linear DNA fragments both the duplex end invasion and also invasion of (dCA)10 into the internal repetitive regions of the duplexes were demonstrated. Then (dTG)10-oligonucleotide invasion into the linear duplexes was demonstrated at molar ratios 50 times lower than that for (dCA)10-oligonucleotide. The phenomenon of duplex-oligonucleotide interaction was also demonstrated for circular DNA plasmid containing CA-repeats. Moreover, it was found that both (dCA)10 and (dTG)10 invaded preferentially supercoiled form of the plasmid, when supercoiled and linearized plasmids were in mixture. Results of this study propose some structural lability of canonical B-form DNA in CA/TG-repeats with formation of single-stranded DNA region.

A common SNP of MCPH1 is associated with cranial volume variation in Chinese population. *B. Su[1,2], JK. Wang[1,2,4], Y. Li[3]* 1) Kunming Inst Zoology, Chinese Academy Sciences, Kunming, Yunnan, China; 2) Kunming Primate Research Center, Chinese Academy of Sciences, Kunming, Yunan,China; 3) Department of Biochemistry, Qujing Normal University, Qujing, Yunnan, China; 4) Graduate School of Chinese Academy of Sciences, Beijing, China.

The microcephaly genes are informative in understanding the genetics and evolution of human brain volume. MCPH1 and ASPM are the two known microcephaly causing genes that were suggested undergone recent positive selection in human populations. However, previous studies focusing only on the two tag SNPs of MCPH1 and ASPM failed to detect any correlation between gene polymorphisms and variations of brain volume and cognitive abilities. We conducted an association study on eight common SNPs of MCPH1 and ASPM in a Chinese population of 867 unrelated individuals. We demonstrate that a nonsynonymous SNP (rs1057090, V761A in BRCT domain) of MCPH1 other than the two known tag SNPs is significantly associated with cranial volume in Chinese males. The haplotype analysis confirmed the association of rs1057090 with cranial volume, and the homozygote males containing the derived alleles of rs1057090 have larger cranial volumes compared with those containing the ancestral alleles. No recent selection signal can be detected on this SNP, suggesting that the brain volume variation in human populations is likely neutral or under very weak selection in recent human history.

Towards a revised Ghent nosology for the Marfan syndrome. *B. Loeys[1], B. Callewaert[1], J. De Backer[1], L. Faivre[2], G. Jondeau[3], R. Devereux[4], R. Pyeritz[5], P. Sponseller[6], P. Wordsworth[7], D. Milewicz[8], H. Dietz[6], A. De Paepe[1]* 1) Dept Medical Genetics, Ghent Univ Hosp, Gent, Belgium; 2) CHU Dijon, France; 3) AP-HP, Hopital Bichat, Paris, France; 4) Weill Medical College of Cornell University, New York; 5) University of Pennsylvania, Philadelphia; 6) Johns Hopkins University, Baltimore; 7) University of Oxford, Oxford, UK; 8) University of Texas, Houston.

The diagnosis of Marfan syndrome (MFS) relies on a set of international criteria, outlined by expert opinion. In 1996, the initial Berlin nosology was revised because of the risk of overdiagnosis and redefined as the Ghent nosology, a more stringent set of major and minor criteria. These Ghent criteria have proven to work well since with improving molecular techniques, confirmation of the diagnosis is possible in over 95% of patients. However, concerns with the Ghent criteria are that some of the diagnostic manifestations have not been validated as hinge points (eg. dural ectasia) and others necessitate cumbersome imaging studies. Moreover, in the absence of aortic dilation, the diagnosis can be stigmatizing, hamper career aspirations and restrict life-insurance opportunities. The label MFS may cause psychosocial burden by restricted exercise permission and situational depressions. Following an international expert meeting, we propose a revised Ghent nosology in which aortic root aneurym and ectopia lentis are cardinal features. In absence of any family history, the presence of these two manifestations is sufficient for the unequivocal diagnosis of MFS. In absence of any of these two, the presence of bonafide FBN1 mutation or a combination of systemic features is required. For the latter a new scoring system has been designed and validated. In this way FBN1 testing is not mandatory but useful when available. The proposed new nosology puts more weight on the cardiovascular manifestations of the disease. We anticipate that the new nosology can delay a definitive diagnosis of MFS but decreases the risk of premature or mis-diagnosis and facilitates discussion of risk and follow-up / management guidelines.

A New Recurrent Translocation with 3:1 Meiotic Non-Disjunction: The Palindrome Mediated t(8;22)(q24.13;q11.21). *M. B. Sheridan[1], C. Haldeman-Englert[1], G. R. Jalali[1], J. M. Milunsky[2], Y. Zou[2], R. Klaes[3], A. M. Hacker[1], J. Brown[1], D. Tomkins[1], T. H. Shaikh[1,4], E. H. Zackai[1,4], B. S. Emanuel[1,4]* 1) Children's Hospital of Philadelphia, Philadelphia, PA; 2) Boston University School of Medicine, Boston, MA; 3) Zentrum f. Humangenetik Mannheim, Mannheim, Germany; 4) University of Pennsylvania School of Medicine, Philadelphia, PA.

Several genetic disorders are associated with rearrangements of chromosomal region 22q11.2, such as translocations which give rise to the recurrent t(11;22) malsegregation-derived supernumerary der(22)t(11;22) syndrome. Cloning and sequence analysis of junction fragments from numerous different translocation carriers has indicated that these recurrent 22q11.2 breakpoints (BPs) occur at the center of a nearly perfect palindromic AT-rich repeat (PATRR). A patient carrying a supernumerary der(22) derived from a 46,XY,t(8;22)(q24.1;q11.2) rearrangement was recently reported. The BPs were mapped and found to occur within PATRRs on both 8q and 22q. We have identified a second patient carrying a similar supernumerary der(22). A comprehensive search of the literature has revealed at least eight prior reports of this translocation. Several of these reports indicated gross molecular positioning of the 8q and 22q BPs consistent with what would be predicted based on our two cases. We subsequently received samples from two of these cases and have performed additional analyses. As predicted, the BPs are identical by FISH, PCR plus sequencing, or array analysis. Preliminary data from PCR analysis of two sperm samples suggests that in some normal males the t(8;22) arises during gametogenesis, similar to the t(11;22). Thus, we have discovered a new recurrent translocation that involves 22q11.2. Like the t(11;22), this translocation can malsegregate 3:1 to produce unbalanced karyotypes and involves identical BPs within PATRRs on both 8q and 22q. The existence of at least twelve cases of this rearrangement provides an opportunity to examine additional aspects of recurrent chromosomal rearrangements involving 22q11.2 and to begin to further evaluate the mechanisms and factors that are likely to produce such rearrangements.

Identification of rearrangements in causative genes of hereditary spastic paraplegia based on a high-resolution array comparative genomic hybridization method. *H. Ishiura, Y. Takahashi, J. Goto, S. Tsuji* Dept Neurology, Univ Tokyo, Tokyo, Japan.

[Objective] A high-resolution array comparative genomic hybridization method (aCGH) was developed to allow efficient detection of rearrangements including deletions/duplications of the causative genes of hereditary spastic paraplegia (HSP). [Background] HSP is a neurodegenerative disorder characterized clinically by progressive lower limb spasticity and pyramidal weakness. To date, as many as 18 causative genes have been identified. Since large rearrangements have been found at least in *spastin*, *paraplegin*, *REEP1*, *atlastin*, and *PLP1*, development of a high throughput system allowing detection of these rearrangements is required. We developed a high-resolution aCGH system to allow simultaneous detection of gene rearrangements of many causative genes for HSP. [Method] We designed a custom made array for CGH (Agilent) to detect genomic rearrangements in 16 causative genes of HSP which included *L1CAM* (SPG1), *PLP1* (SPG2), *atlastin* (SPG3A), *spastin* (SPG4), *CYP7B1* (SPG5A), *NIPA1* (SPG6), *paraplegin* (SPG7), *strumpellin* (SPG8), *KIF5A* (SPG10), *spatacsin* (SPG11), *HSP60* (SPG13), *BSCL2* (SPG17), *spartin* (SPG20), *maspardin* (SPG21), *REEP1* (SPG31), and *ZFYVE27* (SPG33) in 8X15K format (average length between probes: 197bp). Eighty-six HSP patients in whom a comprehensive resequencing analysis of the causative genes did not detect any mutations were enrolled; 21 with ADHSP, 12 with ARHSP, 7 with unknown mode of inheritance, and 46 sporadic patients. [Results] Five rearrangements in *spastin* were identified; 3 deletions and 1 duplication in ADHSP (4/21=19%) and 1 deletion in a patient with a possibly affected sibling. Genomic rearrangements in genes other than *spastin* were not detected. In our previous studies, we have identified 23 mutations of *spastin* in 44 ADHSP (23/44=52%). Taken together, the present study revealed that the overall proportion of SPG4 in ADHSP were 61% (27/44). [Conclusion] Our comprehensive and high-resolution aCGH analysis system successfully detected genomic rearrangements. The proportion of SPG4 in ADHSP in our population was considerably higher than previous reports.

Mothers' Attitudes toward Collection of DNA for Gene-Environment Studies. *M. M. Jenkins[1], E. Reed-Gross[2], W. D. Barfield[1], C. E. Prue[1], M. L. Gallagher[1], S. A. Rasmussen[1], M. A. Honein[1]* 1) Centers for Disease Control and Prevention, Atlanta, GA; 2) Westat, Rockville, MD.

To assess attitudes toward DNA collection, focus groups were conducted with Atlanta mothers who had participated in the National Birth Defects Prevention Study, a multi-site case-control study of birth defects. Within 3 years of completing the interview and being mailed a kit containing cytobrushes for buccal cell collection from themselves, their child, and the childs father, mothers were recruited. Focus groups were segmented based on participation in DNA collection (participators/non-participators), mothers race/ethnicity, childs birth defect status (case/control), and childs birth weight. 3 groups of participators and 3 groups of non-participators each included 1 group of African-American mothers of case-infants, 1 group of mothers of any race/ethnicity who had case-infants of low birth weight, and 1 group of mothers of any race/ethnicity who had control-infants. 8-10 women attended each participator group and 2-5 women attended each non-participator group. Moderator-led discussions probed mothers attitudes toward providing DNA, factors that affected their decision to participate in DNA collection, and preferred collection methods. Non-participators voiced concerns about their DNA being used for purposes they had not agreed to (e.g., cloning or paternity testing) and were especially concerned with government access to their DNA. Information provided (or not provided) on DNA use, storage, and disposal influenced decision-making. Many women reported that fathers skepticism was a significant barrier to participation. Women were asked to rank options for DNA collection (cytobrushes, saliva, mouthwash, blood spots, and whole blood). Attitudes toward whole blood collection for DNA were overwhelmingly negative. Methods that were convenient and non-invasive were preferred. Non-participators expressed concerns about sterility of DNA collection materials and were less comfortable with self-collection methods. Better understanding attitudes toward DNA collection might reduce risk of racial disparities in participation and benefit future studies of gene-environment interaction.

Differential Expression of Dendritic Cell Immunoregulatory Receptor. *M. Ronninger, C. Eklöw, J. C. Lorentzen, L. Klareskog, V. Malmström, L. Padyukov* Department of Medicine, Karolinska Inst & Hosp, Stockholm, Sweden.

Accumulating evidence from human rheumatoid arthritis (RA) and experimental animal studies suggest that Dendritic Cell Immunoregulatory Receptor (DCIR) is an important etiological factor in development of autoimmunity, specifically in RA. Previously we have described the association of variations in the human *DCIR* gene with the level of expression of mRNA isoform 4, which lack the transmembrane domain sequence. To investigate the distribution and regulation of *DCIR*, we analyzed *DCIR* mRNA expression in peripheral blood mononuclear cells (PBMC), synovial fluid mononuclear cells and in synovial tissue using real time RT-PCR. The protein expression was determined by flow cytometry and immunohistochemistry. Results were analysed together with extensive SNP genotyping data across the locus. We confirmed association of SNP rs204301 with expression of *DCIR* mRNA isoform 4 in PBMC from an independent cohort of 20 asthma patients and 20 healthy controls analysed together ($p=0.002$, Kruskal-Wallis test). The mRNA expression of *DCIR* isoforms detected in PBMC from RA or asthma patients was however not different in comparison with healthy controls. DCIR protein was not detected in synovial tissue from healthy donors, while it was abundantly expressed in synovial tissue from RA patients. In PBMC a predominant expression of isoform 1 was detected, while in DCIR-positive T-cells from synovial fluid an equal level of expression of *DCIR* isoform 1 and 4 was observed. Our data demonstrate that genetic variations may regulate expression of *DCIR* isoforms and a pronounced expression of putative soluble isoform of DCIR in T cells from rheumatic joints. We suggest such a soluble variant of DCIR may play a role in immunological regulation and in the development of autoimmunity.

DNA copy number variation of a leptin receptor gene locus associates with symptoms of metabolic syndrome. *S. Shim, J. Jeon, H. Nam, G. Ryu, J. Lee, Y. Cho, T. Chung, J. Kim, H. Kim, B. Han* Korea BioBank, Center for Genome Sciene, Korea NIH, Seoul, Korea.

Structural genomic variations are frequent and widespread in healthy individuals. Recent efforts have been made to link some complex human traits and disease susceptibilities to gains or losses of DNA copy numbers in the genome. In an attempt to identify DNA copy number variations (CNVs) associated with type 2 diabetes mellitus (T2DM) and metabolic syndrome, we initially selected potential candidate genes (n=12) which exhibited DNA copy number variations among Koreans according to SNP chip data. DNA copy numbers were determined by the QMPSF method in blood DNA from community based cohort samples (n=1342). Our candidate gene approach led to identify a biallelic copy number polymorphism of an intronic sequence at leptin receptor (LERP) gene locus. The high copy number allele of the LEPR gene locus was associated with a lower fasting glucose level among nondiabetic subjects ($p < 2.4 \times 10^{-9}$ in men and $p < 2.4 \times 10^{-7}$ in women). The association of total cholesterol with the high copy number allele was also found in among nondiabetic subjects while the association was stronger in men ($p < 2.4 \times 10^{-8}$) than women ($p < 0.019$). In addition, the significant associations between fasting and postprandial insulin levels and copy number variation of LEPR gene locus were evident in only nondiabetic women ($p < 0.004$ and $p < 0.0004$, respectively). This observation indicates that a structural variation at the LEPR gene locus is associated with symptoms of metabolic syndrome.

Outcome of prenatal screening among Ashkenazi Jewish carrier couples for the common non-neuronopathic genotype of Gaucher disease. *Y. Eitan, A. Abrahamov, M. Phillips, D. Elstein, A. Zimran* Gaucher Clinic, Shaare Zedek Medical Center, Jerusalem, Israel.

Background: The aim of elective pre-marital/prenatal carrier testing is to identify couples at risk of having offspring with serious autosomal recessive disorders. Carrier screening for Gaucher disease among Ashkenazi Jews in whom there is a predilection for the non-neuronopathic form of the disease has been available in Israel since 1995, but is controversial because this form is not lethal, is often asymptomatic, and, should signs and symptoms require specific intervention, has effective and safe treatment. The purpose of this study was to evaluate the outcome of counseling given to carrier couples of a fetus with the common non-neuronopathic Ashkenazi Jewish genotype, N370S/N370S. Methods: The records of all couples who were seen for individualized prenatal consultation with a Gaucher expert and whose fetus was N370S/N370S were reviewed to ascertain the fate of their child and whether symptomatic disease was noted during long-term follow-up. Results: Among 38 carrier couples who requested consultations over 12 years, 12 children (4 girls) with the N370S/N370S genotype were seen during the past 9 (range: 0.8-8.5) years. Of these, 8 children had no signs or symptoms attributable to Gaucher disease; 4 children had some questionable sign or symptoms. No child had signs or symptoms that were definitively due to Gaucher disease. No child required enzyme replacement therapy. Three pregnancies were aborted. Conclusion: Consultations with Ashkenazi Jewish couples carrying a N370S/N370S fetus should be aimed at relieving anxiety as the majority of children will probably be asymptomatic. Policies for establishing appropriate criteria for inclusion in screening panels may be useful to the Ashkenazi Jews and to clinicians so as not to instill unwarranted concerns.

Subcellular localization of UDP-GlcNAc 2-epimerase/ManNAc kinase in cells of HIBM and sialuria patients. *K. Patzel, C. Ciccone, I. Manoli, D. Adams, D. Krasnewich, WA. Gahl, M. Huizing* MGB, NHGRI, NIH, Bethesda, MD.

UDP-GlcNAc 2-epimerase/ManNAc kinase (GNE/MNK) is a rate limiting, bifunctional enzyme in sialic acid (SA) synthesis, which is feedback inhibited by CMP-SA in its allosteric site. Mutations in GNE/MNK cause two human disorders, Hereditary Inclusion Body Myopathy (HIBM) and sialuria. Sialuria, clinically characterized by variable symptoms, including hepatomegaly and mental retardation, is caused by dominant mutations in the allosteric site of GNE/MNK, leading to a loss of feedback-inhibition and increased excretion of SA. HIBM is characterized by adult onset progressive muscle wasting and results from recessive, mostly missense, mutations in GNE/MNK outside the allosteric site, leading to decreased GNE/MNK enzyme activities, and decreased SA production. Recent immunofluorescence studies showed that GNE/MNK not only resides in the cytoplasm and Golgi, as expected, but also in the nucleus. This nuclear localization is remarkable, since GNE/MNK performs its enzymatic role within the cytoplasm. However, GNE/MNK contains a putative nuclear export signal and the nucleus contains CMP-SA synthase, which converts all cellular SA to CMP-SA. We propose that GNE/MNK is stored in the nucleus, in its inactive, CMP-SA bound, feedback-inhibited form. If the cell needs SA, there is less SA to enter the nucleus and form CMP-SA, so there is more free GNE/MNK to translocate to the cytoplasm (through its nuclear export signal) and produce more SA. To study this proposed mechanism, we obtained preliminary data using normal, HIBM and sialuria patients fibroblasts. Pilot immunofluorescence studies revealed a higher nuclear GNE/MNK content in sialuria cells and a lower content in HIBM cells compared to normal. Subcellular fractionation followed by Western blotting revealed similar results; higher nuclear amounts of GNE/MNK in sialuria and lower amounts in HIBM. In addition, Western blots of nuclear fractions of both sialuria and HIBM cells showed unexplained GNE/MNK bands compared to normal. These bands, as well as the function of the nuclear export signal, are being analyzed and may provide intriguing insights into the regulation of cellular SA synthesis.

Identification of a RPGR mutation in a male sporadic patient presumed to be affected with Stargardt disease. *J. Kaplan[1], N. Delphin[1], I. Ghazi[2], A. Munnich[1], J.-L. Dufier[2], O. Roche[2], J.-M. Rozet[1]* 1) Genetics, INSERM U781 & Université Paris Descartes, Paris, France; 2) Dpt Ophthalmology, Hôpital des Enfants Malades, Paris, France.

Purpose: The aim of the study presented here was to identify the disease mutation in a sporadic male patient reported to be affected with autosomal recessive Stargardt disease. **Patient and Method:** A young man without familial history came to the genetic counselling consultation of the Hopital Necker in Paris to know the risk for his offspring to be affected with Stargardt disease (STGD). Indeed, he presented with a sudden loss of visual acuity at the age of 24. Ophthalmoscopic and angiographic examinations showed a bulls eye aspect of the macula but no dark choroid and no yellowish perimacular spots. Abnormal colour vision with a green-red confusion axis was noted as well as a central scotoma at the visual field recordings. Nevertheless, he wore corrective glasses for high myopia (OD -11; OG -14) since the age of 3 while his mother presented with a highly asymmetrical myopia (OD -9.75; OG -0.75) resulting in a moderate amblyopia. Recently, electroretinogams of the patient showed a severe alteration of the photopic responses. These last findings in a male sporadic case and in his mother prompted us to challenge the diagnosis of STGD and to consider that of X-linked cone dystrophy. Subsequently, the 3 end of the ORF15 of the RPGR gene, known to contain all cone dystrophy-associated mutations, was screened by direct sequencing. **Results:** The hypothesis of the involvement of RPGR in this family was confirmed by the identification in the proband's DNA of a frameshift mutation in the ORF15 exon of the gene (c.delA1523, p.Lys507Lysfs+3X). Up to date, his mother did not accept to be genotyped. **Conclusion:** This observation highlights the necessity to be particularly cautious when making the diagnosis of STGD in young male patients presenting with the bulls aye phenomenon only. A careful interview of the parents and the follow-up of the patient may direct towards the diagnosis of X-linked cone dystrophy that can easily be confirmed by the restricted screening of the 3 region of the RPGR-ORF15.

A single base transition is the likely cause of persistent Mullerian duct syndrome (PMDS) in a canine model. *S. Pujar[1], X. Wu[3], S. Wan[3], M. M. Lee[3], M. E. Haskins[4], D. H. Schlafer[2], V. N. Meyers-Wallen[1]* 1) J A Baker Institute for Animal Health, Cornell University, Ithaca NY, 14853; 2) Department of Biomedical Sciences, College of Veterinary medicine, Cornell University, Ithaca, NY, 14850; 3) Pediatric Endocrine Division, Department of Pediatrics and Cell Biology, University of Massachusetts School of Medicine, Worcester, MA, 01655; 4) Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA 19104.

Mullerian ducts, precursors of the fallopian tubes, uterus, cervix and cranial vagina in mammals, are present in male and female embryos at the sexually indifferent stage. Later, fetal testes produce Anti-Mullerian hormone (AMH), which binds to its specific type 2 receptor (AMHR2) and leads to Mullerian duct regression. Persistent Mullerian duct syndrome (PMDS) results from failure of Mullerian duct regression in XY males. Approximately 85% of human PMDS cases are caused by mutations in *AMH* or *AMHR2*. The canine model was studied to identify additional mutations underlying PMDS. Canine PMDS, known in miniature schnauzer (USA), bears striking resemblance to the human disease. In the model, derived from the miniature schnauzer, testicular AMH expression and bioactivity are present in affected embryos during the critical period for Mullerian duct regression. The target organ defect could lie in any effector downstream of AMH, including AMHR2. Individuals from the canine PMDS pedigree subset were screened for mutations in the *AMHR2* coding region. Sequencing revealed a C to T transition in *AMHR2* that was concordant with the PMDS phenotype. All affected males were homozygous for the mutation, while normal males were either heterozygous or homozygous for the wild type allele. The mutation introduces a premature stop codon, which likely results in a truncated protein that contains only the extracellular domain, similar to the human mutations causing PMDS. The canine model may provide further insights from correlation between clinical phenotype and the molecular defect.

Evidence that different genes influence bone metabolism at different ages. *C. Kammerer[1], J. Shaffer[1], J. Bruder[2], S. Cole[3], T. Dyer[3], L. Almasy[3], J. MacCluer[3], J. Blangero[3], R. Bauer[2], B. Mitchell[4]* 1) U Pittsburgh, Pittsburgh, PA; 2) U Texas Health Science Center, San Antonio, TX; 3) Southwest Foundation for Biomedical Research, San Antonio, TX; 4) U Maryland, Baltimore, MD.

Low bone mineral density (BMD) is the principal risk factor for osteoporosis and a cause of hip fracture in women and men. Bone metabolism changes over the lifespan, with peak BMD acquisition occurring during the 3rd or 4th decade and BMD loss occurring thereafter and accelerating with age. Although peak BMD and BMD loss are heritable, genetic studies have identified and replicated few genes influencing BMD variation, possibly due to genetic heterogeneity among populations of differing ancestry, age, and recruitment criteria. To explore the hypothesis that different genes influence determinants of BMD during different phases of bone metabolism, we performed genome-wide linkage analysis on 3 measures of the BMD age-trajectory, (1) peak BMD, (2) early BMD loss, and (3) late BMD loss. Femoral neck (FN) and total hip (TH) BMD were measured at two time points (5.6 years apart) in members of 34 extended Mexican American families, from which peak BMD (in all participants; n=884), early BMD loss (in those <45 years; n=337) and late BMD loss (in those >45 years; n=300) were calculated. Heritability of peak FN BMD, early loss, and late loss were 0.57, 0.22, and 0.00, respectively. Heritability of peak TH BMD, early loss, and late loss were 0.55, 0.34, and 0.44, respectively. Linkage analyses provided evidence of a quantitative trait locus (QTL) on chromosome 2p in men (LOD=3.04) for peak FN BMD, but not for early or late FN BMD loss. Likewise, evidence of a QTL for early FN BMD loss, but not peak FN BMD or late FN BMD loss, was observed on chromosome 1q (LOD=3.6). Suggestive evidence of a QTL for peak TH BMD (LOD=2.9) in men, but not early or late TH BMD loss, was observed on chromosome 13q. These results indicate that genes influencing peak BMD, early BMD loss, and late BMD loss may differ. Moreover, analyzing trajectory parameters for other traits that change over time, such as cardiovascular disease, may clarify conflicting results and facilitate identification of genes influencing disease.

Is there genetic heterogeneity of celiac disease among populations? *J. Romanos[1], D. Barisani[2], G. Trynka[1], A. Zhernakova[3], M. T. Bardella[4,5], C. Wijmenga[1]* 1) Dept. Genetics, UMC Groningen, University of Groningen, the Netherlands; 2) Dept. Experimental Medicine, University of Milano Bicocca, Italy; 3) Complex Genetics Section, DBG-Dept. Medical Genetics, UMC Utrecht, the Netherlands; 4) Fondazione IRCCS Ospedale Maggiore Policlinico, Mangiagalli e Regina Elena, Milan, Italy; 5) Dept Medical Sciences, University of Milan, Italy.

Celiac disease (CD) is an inflammatory disorder of the small intestine triggered by gluten peptides and we are studying the involvement of susceptibility genes for CD in different populations. Allelic variants of the HLA-DQ locus (HLA-DQ2/DQ8) contribute to ~40% of CD etiology, while other genes, like *MYO9B*, *CTLA4*, *PARD3* and *MAGI2*, have a modest effect. Most of these minor genes show heterogeneity within European populations since they were not replicated in all studied populations. In southern Europe, individuals carrying DQ2.5 trans are more prevalent than in the north, where individuals carry DQ2.5 cis. Recently, the first genome-wide association study (GWAS) on CD and its follow-up identified 8 new loci that contribute significantly towards CD risk in UK (2 cohorts), Dutch and Irish populations. Seven of these contain genes controlling adaptive immune responses, including *IL2/IL21*, *RGS1*, *IL18RAP*, *CCR3*, *IL12A*, *TAGAP* and *SH2B3*. We selected the 9 most associated single nucleotide polymorphisms to tag the 8 new loci in an Italian cohort composed of 538 CD cases and 593 healthy controls. Common variation in *IL2/IL21*, *RGS1*, *IL12A/SCHIP*, *LPP*, *TAGAP* and *SH2B3* loci was associated with susceptibility to CD in our Italian cohort, but we were unable to detect association between the *CCR3* or the *IL18RAP* loci and CD. This could be due to a low power to detect an OR = 1.2, but it might also be due to genetic heterogeneity among populations. We looked at the separate results of the 4 populations analyzed for the GWAS and noticed that not all of the loci were associated in all of the cohorts. This is the first replication study of 6 of the 8 new CD loci. It is also the first in a southern European cohort. Our results may imply there is a genuine population difference across Europe regarding the loci contributing to CD.

Modeling the RET gene to search for other genetic factors in Hirschsprung disease. *A. Jannot[1,2], J. Amiel[2], F. Clerget-Darpoux[1], S. Lyonnet[2], the Hirschsprung Disease consortium* 1) INSERM, U535, Villejuif F-94817, France; 2) INSERM, U781, Paris F-75743, France.

The genetic background of Hirschsprung disease (HSCR) involves one major gene, the *RET* protooncogene. Many studies demonstrated that both coding sequence mutations and several polymorphisms at the *RET* locus are associated with the disease risk, but up to now, the *RET* gene has never been modeled with a unified approach. Moreover, other genes are involved in addition to *RET*, but most of them have not been yet identified. The International HSCR Consortium, which gathers French, American, Italian, Dutch, Spanish and Chinese teams, has collected 780 families for whom RET mutations on coding sequence have been searched for and 16 SNPs have been genotyped. Moreover, 220 Caucasian trios from this consortium were characterized on a 500K SNP chip. The first step of this study was to precisely define the role of *RET* in HSCR. We found a geographic genetic heterogeneity, but no sex and phenotypic heterogeneity for *RET* SNPs involvement in HSCR, in contradiction with the results of many studies. Using the combination test (1), we have then identified the variants that are the most associated to HSCR and shown that several common variants are necessary to explain *RET* involvement in the disease. The combination of SNPs that best explained the association was used to estimate genotypic risks and their confidence interval using the MASC method (2), which uses both linkage and association information. This combination of SNPs was also used to analyze the genome-wide data to search for a joined association of *RET* and other genetic factors focusing on *RET* gene pathway and on X chromosome, as the high sex-ratio (4/1) in favor of girls for HSCR remains unexplained. HSCR being regarded as a model for complex diseases, this study promotes a stepwise strategy to model candidate genes and gene pathway and to refine associated risks, a necessary step towards a better understanding of the disease and a personalized genetic counseling. 1. Jannot, A.S. et al (2003). Genet Epidemiol, 25, 158-67. 2. Clerget-Darpoux et al (1988). Ann Hum Genet, 52, 247-58.

Common type 2 diabetes and raised fasting glucose variants reduce size at birth. *R. M. Freathy[1], A. J. Bennett[2], S. M. Ring[3], B. Shields[1], C. J. Groves[2], N. J. Timpson[2,3], A. Pouta[4,5], A. Ruokonen[5], E. Hypponen[6], C. Power[6], P. Elliott[7], D. P. Strachan[8], M.-R. Jarvelin[4,5,7], G. Davey Smith[3], M. I. McCarthy[2], T. M. Frayling[1], A. T. Hattersley[1]* 1) Peninsula Medical School, Exeter, UK; 2) University of Oxford, Oxford, UK; 3) University of Bristol, Bristol, UK; 4) National Public Health Institute, Oulu, Finland; 5) University of Oulu, Oulu, Finland; 6) UCL Institute of Child Health, London, UK; 7) Imperial College London, UK; 8) St. Georges, University of London, UK.

Small size at birth is associated with a higher risk of several common late-onset diseases, including type 2 diabetes (T2D), but the cause of these associations is unknown. The fetal insulin hypothesis proposes that the association might be explained by the inheritance of common variants that reduce insulin secretion/action, thereby predisposing to T2D and also to reduced birth weight, since insulin is a key fetal growth factor. To date, we have examined 6 T2D (*TCF7L2*, *CDKAL1*, *CDKN2A/B*, *HHEX-IDE*, *IGF2BP2*, *SLC30A8*) and 2 fasting glucose (*GCK*, *G6PC2*) loci for their impact on birth weight in upto 7887 mothers and 18958 offspring from 4 studies of white Europeans. Three of the 8 variants were associated with reduced size at birth when carried by the fetus. Each T2D or hyperglycemia risk allele at the *CDKAL1*, *HHEX-IDE* and *G6PC2* loci was associated with a 21g [95%CI:11-31g; $P=2\times10^{-5}$], 14g [95%CI:4-23g; $P=0.004$] and 39g [95%CI:18-60g; $P=0.0002$] lower birth weight, respectively. The *G6PC2* association was only observed when adjusting for maternal genotype, which had an opposing, increasing effect on birth weight, and is 50% correlated with fetal genotype. Each maternal copy of the *G6PC2* variant increased birth weight by 25g [95%CI:4-46g; $P=0.02$]. This is consistent with our previous findings that maternal *TCF7L2* and *GCK* risk alleles are associated with higher offspring birth weight. Our results show how common maternal and fetal genotypes can have competing effects on birth weight. Our data are consistent with the fetal insulin hypothesis and provide the first robust evidence that common T2D and fasting glucose variants can reduce birth weight directly via the fetal genotype.

Identification of Highly Conserved Non-coding Sequences in the Distal Region of 9p. *X. Hauge[1], J. Flowers[1], C. Lese Martin[2]* 1) Dept Biology & Physics, Kennesaw State Univ, Kennesaw, GA; 2) Dept Human Genetics, Emory University School of Medicine, Atlanta, GA.

Deletions of the terminal region of the short arm of chromosome 9 (9p-) are associated with trigonocephaly, dysmorphic facial features, and mental retardation. The majority of deletions in patients encompass cerberus 1 (CER1) at 14.7 Mb of 9p. The heterozygous deletion of the CER1 gene has been postulated to cause trigonocephaly. However, we have previously described a group of patients who have deletions smaller than 12.4 Mb, and thus have an intact CER1 gene. Yet, some of these patients exhibit trigonocephaly. This finding opens the possibility that distant cis-regulatory elements for CER1 gene could reside within the first 12.4 Mb of 9p. Although the identification of regulatory elements is difficult, methods of identifying them include searching for highly conserved non-coding regions across species. However, only a limited number of highly conserved sequences have been characterized for chromosome 9 and none of them are localized in the first 12.4 Mb region of 9p. We systematically examined human sequences in increments of 500 bp in the 10 -12.4 Mb region of 9p, eliminating known gene sequences, and compared these human sequences with those of the mouse and zebrafish using the UCSC Genome Browser. The sequences shared by human and mouse or human and zebrafish were aligned. To date, we have identified 39 human sequences which are at least 75 bp long (ranging from 75 bp to 290 bp) and share 70% identity (ranging from 70-92%) with mouse sequences. We have also identified 15 human sequences which are at least 77 bp long and share 60% identity with zebrafish sequences. A clustering of these conserved non-coding sequences was observed. The knowledge of these highly conserved non-coding sequences will be useful in identifying regulatory elements of the candidate genes for 9p- syndrome.

Pathway analysis of genome-wide association and transcript profile data in autism spectrum disorders. *H. Kilpinen[1,2], K. Rehnström[1,3], J. Saharinen[1,4], L. von Wendt[5], I. Hovatta[1,2], L. Peltonen[1,3,6,7]* 1) Institute for Molecular Medicine, Finland (FIMM) and Dept. of Molecular Medicine, National Public Health Institute, Helsinki, Finland; 2) Research Program of Molecular Neurology, University of Helsinki, Helsinki, Finland; 3) Dept. of Medical Genetics, University of Helsinki, Helsinki, Finland; 4) Genome Informatics Unit (GIU), Helsinki, Finland; 5) Unit of Child Neurology, Hospital for Children and Adolescents, Helsinki, Finland; 6) The Broad Institute, MIT and Harvard University, Cambridge, MA, USA; 7) Wellcome Trust Sanger Institute, Cambridge, UK.

Autism spectrum disorders (ASD) have a strong genetic component, but most of the predisposing variants identified have only a minor effect on the disease. Thus, to identify biological pathways altered in ASDs, alternative approaches that take into account multiple affected genes and pathways simultaneously need to be applied. To initiate such studies in the Finnish ASD study sample we first reduced genetic heterogeneity of our samples by analyzing genetic and transcript profiles from an internal isolate of Finland. 54 ASD probands and matched controls were genotyped using Illumina HumanHap300 BeadChip. Transcript profiles were produced from accessible mononuclear cells of ASD patients and matched controls with Affymetrix Human Genome U133Plus2.0. In order to identify biological pathways different between cases and controls, we applied a custom-made non-parametric pathway analysis algorithm to the association analysis results of the genome-wide SNP data. The goal was to identify autism-related pathways by including also "grey zone" SNPs not reaching genome-wide significance in point-wise analysis. Gene annotation data was obtained from the GeneOntology Consortium. After permutation, most significantly affected pathways were those related to developmental processes and synaptic functioning. We are currently obtaining transcript profile data to identify expression-guided pathways in ASD patients. The aim is to compare affected genes, pathways, and genomic distribution of signals in these two datasets, and pave the way towards system biology based analyses to define the molecular pathology behind autism.

Mutations in DMXL1 gene gives rise to a PWS-like phenotype. *K. Gokhale, B. Kulkarni, ELH. Chin, M. Adam, ST. Warren, M. Hegde* Department of Human Genetics, Emory University, Atlanta, GA.

Prader-Willi syndrome (PWS) is a developmental disorder characterized by mental retardation, infantile, hypotonia, poor suck reflex, growth retardation, and childhood onset of pronounced hyperphagia resulting in morbid obesity. PWS is a classic imprinting disorder with most cases resulting from paternal deletions of 15q11-q13 or maternal uniparental disomy 15. However, not all patients who present with PWS-like phenotype have identifiable chromosome 15 involvement, suggesting genetic heterogeneity. We have recently identified 14 novel missense mutations in the DMXL1 gene on chromosome 5 in 12.3% (14/114) of patients with a PWS-like phenotype who previously tested negative for known chromosome 15 etiologies. Analysis of parental samples from three cases showed these missense mutations to all be de novo. While the function of DMXL1 gene is unclear, it is a member of the highly conserved WD repeat protein family, found in all major eukaryotic taxa. Indeed, all but three mutations (79%) replace an amino acid conserved in DMXL1 from human to yeast. We therefore hypothesize that patients with mutations in the DMXL1 gene may present with a PWS-like phenotype. We have made a knock-out mouse model of Dmxl1 which is currently undergoing behavioral assessment. We are also investigating Rav1, the yeast orthologue of DMXL1, to identify DMXL1 interacting proteins and to model the human mutations. This study suggests a novel genetic disorder resembling PWS due to mutations in the highly conserved DMXL1 gene.

Systematic assessment of the effects of population structure on genome-wide association studies. *H. Xu, V. George*
Dept Biostatistics, Med Col Georgia, Augusta, GA.

Large-scale genome-wide association studies are promising for unraveling the genetic basis of complex diseases. Population structure is a potential problem, the effects of which on genetic association studies are controversial. Systematic quantification of the effects of population structure on large scale genetic association studies is needed for valid analysis of the data and correct interpretation of the results. In this study, we performed extensive coalescent-based simulations of samples with varying levels of population structure to investigate the effects of population structure on large-scale genetic association studies. The effects of population structure are measured by the multiplicative changes of the probability of type I error, which is then correlated with the levels of population structure. It is found that at each nominal level of association tests, there is a positive relationship between the level of population structure and its effects, which could be summarized well with a regression function. It is also found that at a specific level of population structure, its effect on association study increases drastically as the significance level of the test decreases. Therefore in genome-wide association studies, the effects of population structure cannot be safely ignored and must be accounted for with proper methods. This study provides quantitative guidelines to the effects of population structure on genome-wide association studies. The results are important for genome-wide association studies in structured or admixed populations.

Fetal Genotype For The Xenobiotic Metabolizing Enzyme NQO1 Influences Intrauterine Growth Among Infants Whose Mothers Smoked During Pregnancy. *T. Grosser[1], T. Price[2], R. Plomin[2], S. Jaffee[2]* 1) Institute for Translational Medicine and Therapeutics, University of Pennsylvania, Philadelphia, PA; 2) Social, Genetic, and Developmental Psychiatry Centre, Institute of Psychiatry, King's College, London, UK.

Maternal smoking during pregnancy retards fetal growth and depresses infant birthweight. The magnitude of these effects may be moderated by fetal genotype. We investigated the relationships between maternal smoking, fetal genotype, and fetal growth in a large population sample of dizygotic twins. Maternal smoking retarded fetal growth in a dose-dependent fashion. In a subsample of 497 twin pairs whose mothers smoked during pregnancy, a functional polymorphism in the NAD(P)H:quinone oxidoreductase gene (NQO1 Pro187Ser; rs1800566) was significantly associated with fetal growth; within twin pairs, possession of the low-activity Ser allele was associated with greater fetal growth. This effect was strongest among moderate smokers. This is the first demonstration that fetal genotype for an enzyme involved in tobacco smoke metabolism influences intrauterine growth independent of maternal genotype.

Supernumery der(22)t(8;22) syndrome occurs by 3:1 meiotic nondisjunction of a recurrent reciprocal translocation. *E. H. Zackai, M. C. Ciano, B. S. Emanuel* Division of Human Genetics, The Children's Hospital of Philadephia, University of Pennsylvania School of Medicine, Philadelphia, PA.

A 3-year-old male with mild developmental delay, large ears, short first metacarpal, fifth finger camptodactyly and a karyotype of 47,XY,+der(22)t(8:22)(q24.13;q11.21)mat prompted a literature review leading to the definition of a new syndrome. The supernumerary der(22)t(8:22) was found to be secondary to 3:1 meiotic nondisjunction of a site specific parental reciprocal translocation. There are 10 cases of +der(22)t(8:22), individually described in the literature, including two sets of siblings. The site specific balanced translocation in the nine families (including our new case) is paternally derived in three and maternal in five (one case unknown). Nine of the eleven cases are male. Birth weight and subsequent growth has been normal. Dysmorphia has been mild with ear abnormalities including large prominent ears (4/11), preauricular pit (3/11), and atretic ear (1/11). Extremity anomalies were noted in the majority - clinodactyly (6/11), camptodactyly (1/11). Three of the nine males had cryptorchidism. All patients presented with mild developmental delay of milestones. IQ, where available, ranged from 50-70. There are no life-threatening structural abnormalities. The ages of the cases ranged from < 5 years (4), 5-20 years (5), to 20-45 years (2). This, after the t(11;22), represents the second most common recurrent reciprocal non-Robertsonian translocation involving chromosome 22. Through 3:1 meiotic nondisjunction it has also been found to produce a specific new syndrome as a result of the presence of a supernumerary der(22).

Characterization of the molecular overlap between Waardenburg syndrome type 2 and 4. *V. Pingault[1,2,3], F. Dastot-Le Moal[1,3], D. Ente[3], T. Attie-Bitach[4], R. Touraine[5], J. Amiel[4], M. Goossens[1,2,3], S. Marlin[6], N. Bondurand[1,2]* 1) INSERM U841, IMRB, Département de génétique, Equipe 11, Créteil, France; 2) Université Paris 12, Faculté de Médecine, IFR10, Créteil, France; 3) AP-HP, Groupe Albert Chenevier-Henri Mondor, Service de biochimie et génétique, Créteil, France; 4) INSERM U781, Hôpital Necker, AP-HP, Paris, France; 5) CHU-Hôpital Nord, Service de Génétique, Saint Etienne, France; 6) Service de Génétique, Centre de référence Surdités génétiques, INSERM U587, Hôpital Armand Trousseau, France.

Waardenburg syndrome (WS) is a rare (1/40000) auditory-pigmentary disorder that exhibits varying combinations of sensorineural hearing loss and abnormal pigmentation of the hair and skin. Depending on additional symptoms, WS is classified into four subtypes, WS1 to 4. Absence of additional features characterizes WS2. The association of facial dysmorphic features defines WS1 and 3 whereas the association with Hirschsprung disease (aganglionic megacolon) characterizes WS4, also called Waardenburg-Hirschsprung disease. Mutations within the genes encoding *MITF* and *SNAI2* have been identified in 15 % of WS2 cases, mutations within the *PAX3* gene explain almost all WS1 and WS3 cases, whereas mutations of *EDN3*, *EDNRB* and *SOX10* are responsible of 40-60% of WS4. Recently, we described the first *SOX10* deletions in patients presenting with WS2. After the involvement of *PAX3* in WS1 and 3, the identification of *SOX10* mutations in WS2 and 4 further documented the molecular complexity and close relationship that link the different subtypes of WS and invited to fully characterize the molecular overlap between WS2 and WS4. We took advantage of the point mutation detection systems routinely used in the laboratory, but also of other strategies such as QMF-PCR assay and looked for point mutations and rearrangements within the *EDN3/EDNRB* and *MITF/SNAI2* genes in both 30 WS4 and 30 WS2 cases. We identified new mutations within the *MITF*, *EDN3*, and *EDNRB* genes. These comprehensive studies are necessary to fully document the complexity of WS, and show that the molecular overlap between WS2 and 4 mostly involves the *SOX10* gene.

Array-based comparative genomic hybridization identifies high frequency of 15q11-q13 duplications in patients with non-syndromic autism spectrum disorders. *A. Battaglia[1], T. Filippi[1], B. Parrini[1], R. Igliozzi[1], A. Novelli[2], L. Bernardini[2], I. Torrente[2], R. Tancredi[1]* 1) Child NeuroPsychiatry, Stella Maris Inst, Pisa, Italy; 2) CSS-IRCCS, S Giovanni Rotondo Inst Mendel, Roma, Italy.

Autism spectrum disorder (ASD) is a heritable developmental disorder in which chromosomal abnormalities are found at a higher rate than the general population. Amongst those, maternally inherited duplications of chromosome 15q11-q13 have been reported in about 1-3%, and recurrent microdeletions and a reciprocal microduplication at 16p11.2 in about 1% of ASD individuals. To assess the frequency of cryptic chromosomal rearrangements in patients with non-syndromic ASD, we studied 23 patients presenting with non-syndromic ASD, using a DNA microarray constructed from 60-mer oligonucleotides spaced at approximately 75 Kb intervals across the genome. All patients were diagnosed as having ASD (with exclusion of the Rett syndrome) after the administration of the ADI-R, and the ADOS-G. Both instruments assess the symptoms of ASD, including autism, as defined in DSM-IV. In addition, a clinical diagnosis was made by the child psychiatrist evaluating the child, on the basis of presence or absence of DSM-IV criteria of autism. Physical and neurological examinations were performed, with particular attention to growth parameters, to any dysmorphic trait or minor anomaly especially involving the face, limbs, and skin, and to variations in muscle tone or to the presence of involuntary movements. All patients underwent karyotype, molecular Fra-X studies, brain MRI (to exclude the presence of any brain abnormality). Blood was collected on the subjects and on their parents. Parental bloods were banked to determine if any detected anomalies were de novo or inherited. Three clinically relevant rearrangements were identified in 3 (13%) patients: two maternally (5 Mb) and one paternally (300 Kb) inherited duplications of chromosome 15q11-q13. All three patients had autism. No differences could be found in the neuropsychological profile between them and the other 20 ASD patients. These results clearly show that aCGH should be considered to be an essential aspect of the genetic analysis of all ASD patients.

Orofacial Clefts in the National Birth Defects Prevention Study, 1997-2004. *A. E. Genisca[1,2], J. L. Frias[1,3], C. S. Broussard[2], M. A. Honein[2], E. J. Lammer[4], C. A. Moore[2], G. M. Shaw[5], J. C. Murray[6], W. Yang[5], S. A. Rasmussen[2]* 1) Centers for Disease Control and Prevention; 2) The CDC Experience Fellowship; 3) McKing Consulting Corp; 4) Children's Hospital Oakland Research Inst; 5) March of Dimes, California Research Division; 6) Dept of Pediatrics, Univ of Iowa.

Orofacial clefts are one of the most common types of birth defects, but their clinical presentation has not been well described in a geographically diverse U.S. population. We used data from the National Birth Defects Prevention Study (NBDPS), a multi-site, population-based case-control study aimed at identifying genetic and environmental risk factors for major birth defects, to characterize the prevalence and phenotype of nonsyndromic orofacial clefts. Included in the analysis were infants born during 1997-2004 with a cleft lip only (CL), cleft lip with cleft palate (CLP), or cleft palate only (CP). Infants with clefts associated with single-gene disorders, chromosomal abnormalities, holoprosencephaly, or amniotic band sequence were excluded. A total of 3,344 infants with orofacial clefts meeting the NBDPS case definition [including 751 (22%) with CL, 1,399 (42%) with CLP and 1,194 (36%) with CP] were identified among 2,731,853 live births. Prevalence estimates for CL, CLP, and CP were 0.3, 0.5, and 0.4/1,000 live births, respectively. The majority (92% of CL, 85% of CLP, and 79% of CP) were classified as having isolated defects (no unrelated major defects). Among those with specified laterality, about twice as many infants with CLP had unilateral vs. bilateral involvement while for CL there were over 10 times as many with unilateral vs. bilateral involvement. For both CL and CLP, involvement was most often left-sided. About one quarter of CP cases had Robin sequence. In infants with CL and CLP, the most common additional major defects were heart defects, limb defects, and musculoskeletal defects, while defects of the heart, limb, and central nervous system were most common in infants with CP. Better understanding of the clinical presentation and the major defects associated with clefts may help guide clinical care as well as contribute to an improved understanding of pathogenesis.

Analysis of hypouricemic patients with respect to the SLC22A12 (URAT1) gene. *B. Stiburkova[1], M. Hosoyamada[3], K. Ichida[4], I. Sebesta[1,2]* 1) Charles University, 1st Faculty of Medicine, Institute of Inherited Metabolic Disorders, Czech Republic; 2) Charles University, 1st Faculty of Medicine, Institute of Clinical Biochemistry and Laboratory Diagnostic, Czech Republic; 3) Department of Pharmacotherapeutics, Kyoritsu University of Pharmacy, Japan; 4) Department of Pathophysiology, Tokyo University of Pharmacy and Life Sciences, Japan.

Hypouricemia is defined as a serum urate levels less than 2 mg/dl and is related to several purine metabolic disorders. URAT1, encoded by the SLC22A12, is the major urate-anion exchanger in the kidney regulating blood urate levels and is presumed to play the central role in reabsorption of urate from glomerular filtrate. Genetic variations of the SLC22A12 gene are associated not only with idiopathic renal hypouricemia (OMIM 220150) but also with reduced renal urate excretion and with elevated serum uric acid levels. Within the scope of selective screening for inherited metabolic disorders conducted from 1999 till 2006 in our department, serum and/or urine levels of the uric acid were investigated in group of 18 854 patients. Purine metabolites were investigated approximately in 3 000 patients. Hypouricemia was found in 569 cases. From these group were carefully selected suspected patients for molecular analysis of the SLC22A12 gene. Detailed purine metabolic investigation revealed repetitively very low serum uric acid in blood (63 mol/l) and elevation of excretion fraction of UA (43%). No one patients had exercise-induced acute renal failure. Subsequent seq. analysis of SLC22A12 (promotor region, 10 exons and intron-exon boundaries) in the 13 subjects revealed 3 sequence variations in the promotor and 5'-UTR region and 5 sequence variations in exonic regions. 3 heterozygous nucleotide missence transition and one heterozygous and homozygous deletion, yet unpublished, were found. Five patients had SLC22A12 sequence variations: one homozygotes, two compound heterozygotes and two heterozygotes. The function and imunohistochemistry analysis by *in vitro* in *Xenopus* oocytes showed significantly decreased urate transport activity of these SLC22A12 sequence variants. This study was supported by grant MSM0021620806 Czech Republic.

Genome wide array diagnostics by high resolution BAC array CGH and 250k SNP array analysis in 1,300 patients with mental retardation. *N. de Leeuw, R. Pfundt, J. Hehir-Kwa, A. Simons, D. Koolen, I. Neefs, N. Leijsten, T. Machielsen, B. van Bon, H. Yntema, W. Nillesen, T. Kleefstra, J. Veltman, B. de Vries, D. Smeets* Dept Human Genetics, Radboud University Nijmegen MC, Nijmegen, Netherlands.

We evaluate our procedures and findings after genome wide array-based Comparative Genome Hybridisation (array CGH) analysis was implemented in 2003 in our diagnostic cytogenetic laboratory for patients with mental retardation with or without multiple congenital anomalies. DNA samples from 500 patients have been analysed by array analysis using a tiling-resolution array consisting of 32,447 BACs and an additional 800 patients using the 250k Affymetrix SNP array platform. In order to achieve fast and reliable interpretation of the copy number variants (CNVs) detected by genome wide array analysis, research and diagnostic colleagues from various disciplines within our department work closely together. All CNV data are stored for potential future analyses and our clinically relevant CNVs are also made available to others through the ECARUCA database (www.ecaruca.net). In ~13% of patients with a normal karyotype and without subtelomeric imbalances a clinically significant CNV was detected by genome wide array analysis, ranging in size from ~50 kb to 15.7 Mb, excluding one mosaic trisomy 8. In an additional 4% of these patients, CNVs were detected that were inherited from either the father (43%) or the mother (57%). Apart from the detection of several new microdeletion syndromes reported by us and others, so far, many novel genes have been identified. Moreover, confirmation and follow-up experiments using FISH revealed a mosaic aberration in two patients. A cryptic, balanced chromosome rearrangement was identified in the respective parent from each of three other patients, thereby explaining the familial occurrence of the clinical phenotypes. Considering the high additional detection rate of clinically relevant CNVs by genome wide array analysis observed in this study, we recommend to start with array analysis in the diagnostic work-up of patients with mental retardation with or without multiple congenital anomalies.

Testing the genes and pathways behind fetal motoneuron disease as candidate ALS genes. *H. O. Nousiainen[1], H. Laaksovirta[2], H. Kaariainen[1], K. Silander[1], M. Kestila[1], L. Peltonen[1,3,4,5]* 1) Institute of Molecular Medicine and National Public Health Institute, Helsinki, Finland; 2) Department of Clinical Neurosciences, Helsinki University Central Hospital, Helsinki, Finland; 3) Department of Medical Genetics, University of Helsinki, Helsinki, Finland; 4) The Broad Institute, MIT, Boston, MA, USA; 5) The Wellcome Trust Sanger Institute, Hinxton CB10 1SA, UK.

Amyotrophic Lateral Sclerosis (ALS) is a late-onset, rapidly progressing neurodegenerative disease affecting motor neurons. ALS usually leads to complete loss of voluntary muscle movement and death within a few years of the appearance of the first symptoms. The etiology of most ALS cases remains unknown, but mutations in Cu/Zn superoxide dismutase *SOD1* and *TARDBP*, encoding the RNA binding protein TDP-43, have been implicated in a small fraction of cases of both familial and sporadic ALS. Here we have taken a pathway based approach in searching ALS genes triggered by our recent findings on exceptional, early onset motoneuron diseases. We recently identified mutations in a gene encoding the mRNA export mediator protein GLE1 in patients with two forms of fetal lethal motoneuron disease. Interestingly, increasing evidence of the involvement of TDP-43 in mRNA processing has also been emerging recently, implicating that mRNA processing and regulation of localized protein synthesis may be a key mechanism in the development, maturation, and functioning of anterior motoneurons in general, and potentially provide clues of the molecular pathogenesis of ALS. We are currently analyzing a nationwide cohort of 300 Finnish ALS patients including both familial and sporadic cases. We first excluded patients with mutations in SOD1 and are currently performing targeted genotyping of the remaining patient samples for 90 SNPs of critical genes involved in mRNA transport and processing using the Sequenom iPLEX Gold chemistry. The study will potentially provide additional information of the molecular mechanisms behind ALS and increase our understanding of the pathogenesis of this devastating disease.

Putative role for miR-562 in kidney development and cancer. *K. Drake, B. Gopalan, M. Aldred* Genomic Medicine Institute, Lerner Research Institute, Cleveland Clinic, Cleveland, OH.

We have identified a 360 kb minimal region of deletion at chromosome band 2q37.1 in up to 5% of Wilms tumors, strongly suggesting the presence of a tumor suppressor gene that predisposes to this childhood kidney cancer. *DIS3L2*, a putative mitotic control homolog is the only gene within this minimally deleted region. Intron 9 of *DIS3L2* contains a previously uncharacterized micro-RNA, miR-562. Recent studies have revealed that miRNAs have key roles in diverse regulatory pathways, including control of developmental timing, apoptosis, cell proliferation and organ development. miRNAs and their targets seem to form complex regulatory networks. For example, a single miRNA can bind to and regulate many different mRNA targets. Bioinformatic analysis of putative miR-562 targets has identified several genes with established roles in kidney development. *EYA-1* encodes a gene that is essential for cell survival and proliferation in early metanephric development. *EYA-1* has also been shown to be highly expressed in Wilms tumors compared to fetal kidney and mutations of this gene occur in branchio-oto-renal dysplasia syndrome, a syndrome associated with renal abnormalities that range from mild hypoplasia through to the complete absence of the kidneys. Other targets include *MET* a hepatocyte growth factor receptor with known oncogenic activity and *PSEN1* a gene involved in Notch and *Wnt* signaling with a role in renal and urological system development and function. These targets suggest that miR-562 may play a role in fetal kidney development. Using RNase protection assays we have shown that miR-562 has tissue-restricted expression, which is highest in the fetal kidney, present in several kidney derived cell lines and absent in the fetal heart and liver as well as MCF7 cells. Screening of 176 Wilms tumor samples revealed a 19 bp deletion of miR-562 in 3 cases. This polymorphism is also present in the normal population, albeit at a low frequency (0.023) and may be a predisposing factor in the development of Wilms tumor. We are currently undertaking a study of putative miR-562 targets, including *EYA1*, *MET* and *PSEN1*, using luciferase reporter assays.

Structural variation in patients with multiple sclerosis. *R. Rabionet[1], E. Docampo[1], M. García[1], L. Armengol[1], E. Munteis[2], J. E. Martínez[2], F. Matesanz[3], A. Alcina[3], J. Roquer[2], X. Estivill[1,4]* 1) Genes & Disease programme, Centre de Regulació Genòmica and Public Health and Epidemiology Network Biomedical Research Center (CIBERESP), Barcelona, Barcelona, Spain; 2) Neurology Service, Hospital del Mar-IMAS, Barcelona, Catalonia, Spain; 3) Instituto de Parasitologia y Biomedicina "López-Neyra", CSIC, Granada; 4) Universitat Pompeu Fabra (UPF), Barcelona, Spain.

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease that affects the central nervous system. Several studies provide evidence that MS is a complex disorder involving genetic and environmental factors. Several different approaches have been undertaken to elucidate the genetic causes of MS, including linkage and association studies. These have consistently shown association to the major histocompatibility complex (MHC) region, specifically the DR15 haplotype. Other MS genes have been more elusive, with ILR7, CD58 and IL2RA as the most consistent. The aim of this study was to evaluate a possible contribution of genomic structural variation to MS susceptibility. Forty relapsing-remitting MS samples were divided into two pools and comparative genomic hybridization (CGH) against a pool of 50 control samples was performed using Agilent 244K arrays. Initial selection criteria (three consecutive probes with a log2 ratio above 0.29) showed differential hybridization between both groups of cases and controls only at the chromosome 6 MHC region, spanning four to six probes (depending on the pool). In addition, one region in chr1 spanning 4 probes appeared as a CNV only in one of the two pools of MS samples (relapsing-remitting). This region has been further characterized and tested for association in a larger sample size (120 samples), obtaining a p-value of 0.051. Further characterization in an additional sample set of 800 samples is under course. Finally, when considering only one probe, seven regions showed differential hybridization in cases versus controls in both pools and were selected for follow-up by RTqPCR experiments.

Analysis of Copy Number Variation within the Chromosome 4 GABA Region in Autistic Individuals. *D. Hedges[1], H. Cukier[1], M. Rayner[1], D. Ma[1], J. Jaworski[1], P. Whitehead[1], H. Wright[2], R. Abramson[2], J. Hussman[3], J. Haynes[4], M. Cuccaro[1], J. Gilbert[1], M. Pericak-Vance[1]* 1) Miami Institute for Human Genomics, University of Miami, Miami, FL,; 2) University of South Carolina School of Medicine, Columbia, SC; 3) Hussman Foundation, Ellicott City, MD; 4) Center for Human Genetics Research, Vanderbilt University, Nashville, TN.

Autism has a strong genetic component. Studies over the past decade have demonstrated that the underlying genetics are complex. Research suggests an important role for copy number variants (CNVs) in autism risk. The GABRA4 gene lies among a 1.5Mb cluster of GABA-related genes on chromosome 4 (chr4), which include GABRA2,GABRA4 and GABRB1. The chr4 GABR cluster has been implicated in autism patients through cytogenetic alterations as well as association with SNPs in the GABRA4 gene. These data point to GABRA4 and potentially other GABR genes in the cluster as autism candidate genes. We examined the GABR cluster on chr4 for CNVs associated with autism. To assess structural variation, we designed custom 4x44k Agilent CGH arrays across the entire chr4 cluster, spanning the 1.49 Mb region with a density of approximately 1 probe every 40 nucleotides. We evaluated 44 individuals who met DSM IV criteria for an autistic spectrum disorder and 30 control individuals. Results show putative CNVs in thirteen distinct sites within the chr4 GABA region. Eleven sites fell within the GABRB1 gene, one site fell between GABRG1 and GABRA2, and the final site was located between GABRA2 and GABRA4. Five CNVs, all within the GABRB1 gene, were unique to the autism population sampled. Three sites contained duplications, one was a putative deletion, and the remaining site exhibited both. Across all 13 loci, there was a trend towards significance ($p \sim 0.062$) for an increased number of deletions in autism cases vs. controls. The statistical trend in this limited sample size suggests that investigation of CNVs in the chr4 cluster in a larger sample set is warranted. We are validating CNVs detected via standard and real-time PCR methods and testing the GABR cluster by CGH on an expanded population size of 250 autistic probands and 50 controls individuals.

Serum uric acid concentrations and sequence variants in methylenetetrahydrofolate reductase (MTHFR)gene. *I. Sebesta[1,2], M. Sedova[3], B. Stiburkova[1], M. Pavlikova[3], J. Zvarova[3], V. Kozich[1]* 1) Institute of Inherited Metabolic Disorders; 2) Institute of Clinical Biochemistry and Laboratory Diagnostics, First Faculty of Medicine, Charles University in Prague; 3) Department of Medical Informatics, Institute of Computer Science of the Academy of Sciences of CR, v.v.i., Czech Republic.

Recent data suggest that an elevated serum uric acid (UA) concentration may play a role in the development of cardiovascular, renal disorders and of metabolic syndrome. We investigated biochemical, anthropometric, and genetic determinants of serum UA levels including the MTHFR sequence variants in the group of 569 healthy subjects (275 men and 294 women). Methods: The association of biochemical, anthropometric, and genetic variables with the UA levels was evaluated in both univariate and multivariate linear regression models. Variables that were significantly associated with UA levels in univariate analysis were used as a set of initial predictors in the backward selection procedure to find the model which best explains the variability of UA levels. Results: The median UA concentration was 5.5 mg/dL for men and 3.8 mg/dL for women. The univariate analysis revealed that individuals homozygous for the c.1298A>C MTHFR variant had significantly reduced UA concentration. In average, individuals with C/C genotype had 0.3 mg/dL lower UA compared to the group of individulas with A/A or A/C genotype. On the other hand, the c.677T>C variant in the MTHFR gene had no significant effect on UA levels. The final model explained variability of serum UA concentration with R2 =0.5721 and included: age, sex, BMI, alcohol consumption, presence of hypertension, c.1298A>C MTHFR variant and concentrations of cystatine, creatinine, hemoglobin, cysteine, triglycerides and activity of enzyme GGT. Conclusions: Our results did not confirm the role of common c.677T>C variant in MTHFR gene as a determinant of hyperuricemia. In contrast, homozygosity for the c.1298A>C MTHFR variant was associated with reduced serum UA concentration. Supported by grant VZMSM0021620806.

Disruption of highly conserved, very distant regulatory elements on either side of *SOX9* is associated with Pierre Robin sequence. *S. Benko[1], JA. Fantes[2], J. Amiel[1], DJ. Kleinjan[2], S. Thomas[1], J. Ramsay[2], N. Jamshidi[3], A. Essafi[2], CT. Gordon[3], C. Golzio[1], N. Kilpatrick[3], P. Thomas[3], M. Vekemans[1], ND. Hastie[2], A. Munnich[1], A. Pelet[1], PG. Farlie[3], DR. FitzPatrick[2], S. Lyonnet[1]* 1) INSERM U781, Université Paris Descartes, Hôpital Necker, Paris, France; 2) MRC Human Genetics Unit, Edinburgh EH4 2XU, UK; 3) Murdoch Childrens Research Institute, Royal Childrens Hospital, Parkville, Australia.

Most evolutionarily conserved human DNA has no protein-coding function but may have regulatory functions whose mechanism and size of the domains controlled remains largely unknown. We show here that disruption of very distant cis-regulatory highly conserved non-coding elements (HCNE) on either side of the *SOX9* gene is associated with Pierre Robin sequence (PRS), an orofacial cleft anomaly with mandibular hypoplasia. We demonstrate the existence of a PRS locus at 17q24 through linkage analysis in a large PRS family, mapping of 3 independent translocation breakpoints that cluster 1.06-1.23 Mb upstream of *SOX9*, and identification of microdeletions containing HCNEs immediately upstream of these breakpoints, or at a similar distance downstream of *SOX9*. The pattern of histone modifications associated with both the centromeric and telomeric regions suggests tissue-specific enhancer function. A heterozygous point mutation was identified in an additional PRS family within one of the upstream deleted HCNEs, capable of driving mandibular expression in transgenic mouse embryos. ChIP experiments demonstrated that this HCNE binds MSX1 protein and the human mutation both alters this binding and abrogates enhancer function in a mandibular mesenchymal cell line. 3D FISH analysis indicates that chromatin decompaction occurs specifically in Sox9 expressing cells within the developing mandible of mouse embryos, suggesting that the enhancer is more likely to be regulating *Sox9* than other flanking genes. Our data, combined with existing evidence from human and animal phenotypes, suggests that the disruption of distant, tissue-specific regulatory elements, required for the normal development of the mandibula, perturbs embryonic expression of *SOX9* and accounts for the PRS phenotype.

Genetic-radiological correlation study in 48 children with characterized genetic mutation responsible for mitochondrial diseases. *A. S. Lebre[1], M. Rio[1], P. de Lonlay[1,2,4], F. Brunelle[3,4,5], A. Rotig[2], A. Munnich[1,2,4], N. Boddaert[3,4,5]* 1) AP-HP, Hôpital Necker-Enfants Malades, Département de Génétique, Paris F-75015 France; 2) Inserm, U781, Hôpital Necker-Enfants Malades, Paris F-75015 France; 3) AP-HP, Hôpital Necker-Enfants Malades, Service de radiologie pédiatrique, Paris F-75015 France; 4) Université Paris Descartes, Faculté de Médecine René Descartes, Paris F-75015 France,; 5) Inserm, U797, CEA, Neuroimagerie en psychiatrie, Service hospitalier Frédéric Joliot, Orsay F-91400 France.

Purpose : Mitochondrial diseases are very complex to study because of the large number of disease-causing genes. For patients with neurological symptoms, we looked for common cerebral imaging pattern according to the type of genetic mutation. Methods used: We report here a retrospective study of cerebral imaging for 48 patients with mitochondrial diseases. Tomodensitometry and Magnetic resonance imaging were usually performed in the Department of Pediatric Imaging. Patients presented mitochondrial diseases with isolated or multiple respiratory chain deficiencies. Causing mutations were identified for all of them. Results : For a same group of mutations, the lesionnal pattern in cerebral imaging was often very similar. For patients with complex I deficiency (n=16), large abnormalities were observed in brainstem (16/16), associated with basal ganglia abnormalities (15/16). Some patients presented sus-tentorials stroke-like (4/16). One of these patients had also an associated leukodystrophy. Patients with complex V deficiency (n=10) presented putamen abnormalities (9/10), associated with cerebellar atrophy in 3 y.o. patients (6/6). Patients with quinone deficiency (n=8) presented with cerebellar atrophy (8/8) and sus-tentorials stroke-like (3/8). Patients with the MELAS mutation (n=7) presented cerebellar atrophy (7/7) and basal ganglia abnormalities. For patients with mutations in POLG gene (n=7), normal imaging was observed in 6/7 patients but 1 patient presented thalamic abnormalities. Our study is the first evidence of a good contribution of cerebral imaging in mitochondrial diseases for orientation of genetic studies and a faster identification of the causing mutations.

Association of polymorphisms in genes related to renin-angiotensin-aldosterone system with left ventricular structure and function and incident heart failure in four ethnic groups; the Multi-Ethnic Study of Atherosclerosis (MESA). *H. Bahrami[1], B. Fang[2], D. A. Bluemke[1], S. Heckbert[3], J. Kaufman[3], J. H. Young[1], X. Guo[2], V. Fernandes[1], D. Herrington[4], S. Shea[5], J. I. Rotter[2], W. Post[1], J. A. Lima[1]* 1) Johns Hopkins Univ; 2) Cedars-Sinai Medical Ctr; 3) Univ. of Washington; 4) Wake Forest Univ; 5) Columbia Univ.

Introduction: Our objective was to determine the associations of the genes encoding the renin-angiotensin-aldosterone (RAA) system with MRI-measured parameters of cardiovascular structure/function and incident CHF in four ethnic groups. **Methods:** 55 tagging SNPs were genotyped in angiotensinogen (AGT), angiotensin converting enzyme (ACE), and angiotensin II receptors 1 and 2 (AGTR1 and AGTR2) genes in 2,847 participants [712 Caucasians (CA), 712 African Americans (AA), 705 Hispanics (HIS) and 718 Chinese Americans (CHN)]. Multiple linear regression was used to determine the associations of these SNPs with LV mass, LV ejection fraction (LVEF), cardiac output, and Cox regression models for CHF, while adjusting for risk factors stratified by racial/ethnic group. Empirical p-values were then calculated for p-values 0.0125 to account for multiple genes tested. **Results:** During follow-up, 38 participants developed CHF. SNPs in AGTR1 [rs422858-C (adjusted HR: 2.9, p:0.011) and rs2638363-A (adjusted HR: 2.4, p<0.001)] were associated with increased risk of CHF in AA; these associations were more pronounced after adjustment for risk factors. SNPs in AGT (rs2478545-A, p:0.002) and AGTR1 (rs275645-G, p<0.001) were independently associated with lower LVEF in CA and HIS, respectively. Lower cardiac output was independently related to polymorphisms in AGTR1 (rs275646-A, p<0.001) in HIS and in AGTR1 (rs4681443-G and rs718858-G, p: 0.002) and AGT (rs3789671-T, p:0.001) in CHN. A SNP in the AGTR2 (rs5950586-A, p:0.0075) was an independent predictor of LV mass in HIS men. **Conclusions:** Polymorphisms in genes encoding RAA system, particularly in AGTR1, appear to be associated with LV function (in HIS & CHN) and incident CHF (in AA). This potential genetic heterogeneity may contribute to ethnic differences in LV function and incidence of CHF.

Associations Between Lactating Womens Vitamin D Levels, Their Breastfed Infants Vitamin D Levels, and VDR Polymorphisms and VDR Expressions in Hair Follicles. *F. Ozkinay[1,2], H. Onay[2], C. Gunduz[3], A. Aykut[1], A. Ekmekci[1], C. Biray[3], H. Akin[2], O. Cogulu[1,2], G. Koturoglu[1], S. Kalkan[1], S. Pehlivan[4], M. Coker[1]* 1) Dept Pediatrics, Ege Univ, Izmir, Turkey; 2) Dept Medical Genetics, Ege Univ, Izmir, Turkey; 3) Dept Medical Biology, Ege Univ, Izmir, Turkey; 4) Dept Medical Biology, Gaziantep Univ, Gaziantep, Turkey.

In human body vitamin D plays a role in the metabolic regulation of a number of systems. Both environmental factors and genetic factors have important influences on vitamin D metabolism. In this study the factors which may effect vitamin D levels in serum and breast milk, the association between vitamin D receptor (VDR) polymorphisms and vitamin D metabolism and VDR expression in hair follicles have been investigated in lactating women and in their 1-6 month-old breastfed babies. The study included 101 lactating women and their 101 exclusively breastfed babies. A questionnaire was applied to the mothers to evaluate their sun exposure, diary vitamin D consumption and diet. The relationships between those environmental factors, six VDR polymorphisms, Cdx2, FokI, BsmI, ApaI, TaqI and Poly A, VDR expression levels in hair follicles and vitamin D metabolites were evaluated. Insufficient sun exposure and vitamin D consumption, low serum 25(OH)D levels were detected in the mothers. The serum 25(OH)D levels of the mothers were well correlated with calcium and vitamin D levels in their milk and their babies' serum 25(OH)D levels. In the condition of normal serum 25(OH)D levels, BB genotype of BsmI polymorphism was significantly associated with low Ca levels in breast milk (p0.04). When serum 25(OH)D levels lower than the normal levels in the mothers, low vitamin D levels (p0.01), in the milk and low VDR expression(p0.04) in the hair follicles were detected, beside low calcium levels in the milk (p0.01). In the mother having high levels of Ca in their milk the frequency of aFBGtS haplotype was significantly higher (p0.02) while both aFBGtS and AfbGTS haplotypes frequencies were significantly higher in mothers having high levels VDR expression in their hair follicles.

Clinical features of Paternal Uniparental Disomy 14 in four related patients with a typical methylation pattern but biparental inheritance of chromosome 14. *G. Morin[1], L. Cuisset[2], A. G. Le Moing[1], B. Demeer[1], A. Receveur[3], J. Andrieux[4], H. Copin[3], M. Mathieu[1]* 1) Clinical Genetic Unit -University Hospital - Amiens - France; 2) Molecular Genetic Laboratory - Cochin Institute - Paris - France; 3) Cytogenetic Unit - University Hospital - Amiens - France; 4) Medical Genetic Laboratory - Jeanne de Flandre Hospital - Lille - France.

Paternal uniparental disomy 14 [upd (14) pat] is responsible of a well recognizable phenotype that includes facial dysmorphism, small bell-shaped thorax, abdominal wall defects, polyhydramnios and developmental delay. Skeletal defects include a distinctive bowing of the ribs with a coat-hanger appearance that gives a good diagnostic clue. So far less than 15 cases with upd (14) pat have been reported. Recently, Kagami et al published 8 additional patients with a paternal upd (14)-like phenotype, but without upd (14). They found deletions of the 14q32.2 region in 4 patients and hypothesized epimutations of this imprinted region in the 4 other patients. Here we report on four patients, a mother and her 3 daughters, born of two separate unions, with clinical features of paternal upd (14). They show a typical methylation pattern of upd (14) pat, but biparental inheritance for chromosome 14. All the classical or molecular cytogenetic investigations were negative suggesting a dominantly transmitted genetic alteration. To our knowledge, this is the first familial report of paternal upd (14)-like phenotype without microdeletion.

13 new patients with 13q deletion syndrome : genotype-phenotype analyses in progress and first report of a Steinfeld syndrome. *C. Quélin[1], C. Bendavid[2,3], C. Dubourg[2,3], C. de la Rochebrochard[1], J. Lucas[4], C. Henry[4], S. Jaillard[2,4], P. Loget[5], L. Loeuillet[6], D. Lacombe[7], JM. Rival[8], V. David[2,3], L. Pasquier[1,2], S. Odent[1,2]* 1) Service de Génétique Médicale, Rennes, France; 2) CNRS UMR 6061 Génétique et Développement, Rennes, France; 3) Laboratoire de Génétique Moléculaire, Rennes, France; 4) Laboratoire de Cytogénétique, Rennes, France; 5) Département dAnatomie et cytopathologie, Rennes, France; 6) Service dAnatomie et Cytopathologie, Poissy, France; 7) Service de Génétique Médicale, Bordeaux, France; 8) Service de Génétique Médicale, Nantes, France.

13q deletion is characterised by a wide phenotypic spectrum resulting from a partial deletion of the long arm of chromosome 13. The main clinical features are mental retardation, growth retardation, craniofacial dysmorphy and various congenital defects. Only one recent Italian study was aimed at determining genotype-phenotype correlations among 13q deletions from a group of mainly live born children, using array-CGH and FISH. In order to improve the molecular characterisation of 13q monosomy, 13 new patients (of which 10 foetuses), were collected based on a cohort of holoprosencephaly linked to ZIC2 gene deletion and/or patients with 13q deletion diagnosed by standard caryotype. First, quantitative gene screening using MLPA was performed to look for ZIC2 gene deletion and then, CGH array analysis was carried out using the Agilent Human Genome CGH microarray 4x44K (Agilent Technologies, Santa Clara, CA). All the foetuses had severe cerebral midline malformations associated with a deletion including the ZIC2 gene. Further candidate genes are suspected to explain the malformations associated with cerebral anomalies in the hypothesis of a contiguous gene syndrome: SPRY2 in 13q31.1 is implicated in lens cell proliferation and differentiation for congenital cataract; GPC5 in 13q32 is mainly expressed in the mesenchyme of the developing limb bud for upper limbs anomalies. Moreover, we reported the first patient with Steinfeld phenotype linked to a chromosomal anomaly, and suggested that some of the associations between cerebral midline malformation and limb defects might be related to a small 13q deletion.

PTCH1 GENE AND RISK OF NON MELANOMA SKIN CANCER AFTER ORGAN TRANSPLANTATION.

A. Begnini[1], M. Gomez Lira[1], G. Tessari[2], L. Naldi[2], G. Remuzzi[2], L. Boschiero[2], A. Forni[2], C. Rugiu[2], S. Piaserico[2], G. Girolomoni[2], A. Turco[1] 1) Dept Mother Child/Biol Genetics, University of Verona, Italy; 2) Dept Biomedical and Surgical Science, Section of Dermatology, University of Verona, Italy.

Organ transplant recipients (OTR) are at higher risk of non melanoma skin cancer (NMSC), particularly basal cell carcinoma (BCC) and squamous cell carcinoma. Risk factors for NMSC in OTR include skin type, UV light exposure, immunosuppression, human Papilloma virus infections, and genetic susceptibility. PTCH1 is a negative regulator of the Hedgehog pathway, that provide mitogenic signals to basal cells in skin. PTCH1 gene mutations cause nevoid basal cell carcinoma syndrome (NBCCS or Gorlin syndrome), and also occur in sporadic forms of BCC. Associations have been demonstrated between PTCH1 polymorphisms and BCC susceptibility in non transplanted recipients. This study was designed to investigate if known polymorphisms of PTCH1 gene contribute to NMSC risk after transplantation and to identify novel genetic polymorphisms in the proximal 5 regulatory region of the gene. We analyzed three PTCH1 gene SNPs (rs2297086, rs2066836 and rs357564) in 302 Northern Italian OTR patients (149 cases and 153 controls). Single locus and haplotype frequencies analysis showed no significant association. We also analyzed a polymorphism involving a GGC trinucleotide repeat located 4 bp upstream of the first methionine codon. No significant difference in allele and genotype frequencies was observed between case and controls. We discovered two novel alleles containing five and six repeats. Screening for polymorphisms was performed by heteroduplex analysis in 30 cases and 30 matched controls. Two polymorphisms, -198A>G and -195G>C, were identified in the 5flanking region. Both variants were in linkage disequilibrium and showed a frequency of 0.005 in 200 tested individuals. Well investigate the effect of the trinucleotide repeat length and of the 2 novel variants on the gene expression using the luciferase reporter gene assay. These results seem to exclude an important role of the PTCH1 gene in NMSC susceptibility after organ transplantation. Further studies on a larger sample are warranted to confirm these findings.

UNC5 genes and breast cancer: LOH analysis and mutation screening. *I. Kurelac[1], E. Bonora[1], C. Evangelisti[1], M. Vargiolu[1], D. Fusco[1], A. Bernet[2], P. Mehlen[2], G. Romeo[1]* 1) Unità di Genetica Medica-Policlinico S. Orsola, Bologna, Italy; 2) CNRS UMR 5238 Laboratoire Apoptose, Cancer et Développement, Centre Léon Bérard, Lyon, France.

Human UNC5 genes encode for membrane receptors that play a role in netrin-1 mediated signaling. They act as mediators of netrin-1 chemorepulsive effect on axon migration during neural development. UNC5 receptors are also considered to be dependence receptors (DR). DRs display the common feature of inducing two completely opposite intracellular signals: in the presence of the ligand they transduce a positive survival signal, whilst in its absence, they induce apoptosis. Expression of human UNC5 genes is down-regulated in multiple types of cancers including breast and colorectal cancers. In the latter, downregulation of UNC5 genes is associated with loss of heterozygosity (LOH). In order to verify whether LOH of UNC5 genes is present also in breast cancers, a panel of 57 normal and tumor breast tissues was analysed using intragenic microsatellite markers. However, no LOH was detected. Since mutations impairing mRNA or protein levels would not have been detected by LOH analysis only, a mutation screening of PCR amplified exons was performed. Four new DNA changes were found in UNC5B and UNC5C genes, three of which were not identified in control samples. All changes result in silent substitutions at the protein level. However, *in silico* analysis predicted that these variants alter the exon splicing enhancer (ESE) binding domains which could modify mRNA processing. A possible pathogenic effect of the variants will be further investigated using a splicing assay with a synthetic minigene. The analysis is currently ongoing and the final results will be presented.

Genome-Wide Association Analysis Identifies Novel Susceptibility Genes for Ulcerative Colitis. *A. Franke[1], T. Balschun[1], T. H. Karlsen[2], S. Schreiber[1]* 1) Institute of Clinical Molecular Biology, Kiel, Germany; 2) Medical Department, Rikshospitalet University Hospital, Oslo, Norway.

Inflammatory bowel disease (IBD) manifests as either of two related subtypes, ulcerative colitis (UC) or Crohn disease (CD), both of which are characterized by diarrhea, abdominal pain and intestinal mucosal inflammation and ulceration. Systematic identification of susceptibility genes for IBD has thus far focused mainly on CD, while little is known about the genetic architecture of UC. Here, we report on a genome-wide association study with 440,794 SNPs genotyped in 1167 UC patients and in 777 healthy controls. The 20 most significantly associated SNPs were tested for replication in three independent European case-control panels comprising a total of 1855 UC patients and 3091 controls. Among the four consistently replicated markers, a SNP immediately flanking a cytokine gene, showed the most significant association in the combined verification samples (P=1.35×10-12; OR=1.46 [1.31-1.62]). Fine mapping of the corresponding region supported the initial finding and confined the disease association. The strongest associations were observed immediately 3 of the gene, in the vicinity of a binding site of the AP-1 transcription factor known to be activated by bacterial lipopolysaccharide. Association between the lead SNP and CD (1848 cases, 1804 controls) was weak (P=0.013; OR=1.17 [1.01-1.34]). Our findings strongly suggest that defective cytokine function is central for the pathogenesis of the UC subtype of IBD. All above mentioned SNPs and susceptibility genes will be reported on the meeting.

Identification of Immortalization Gene Signature of EBV-transformed Lymphoblastoid Cell Lines from a Long-term Subculture Collection. *J. Jeon, H. Nam, S. Shim, T. Chung, J. Kim, B. Han* Korea BioBank, Center for Genome Sciene, Korea NIH, Seoul, Korea.

EBV-transformed lymphoblastoid cell lines (LCLs) are used as a resource for human genetic, immunologic and pharmacogenomic studies. However, there is a limitation of the extensive utilization of LCLs due to possible genetic changes and its relevant gene expression changes during the LCL generation and maintenance. In order to investigate expression phenotype changes during long-term subculture of LCLs, we compared differentially expressed genes from microarray data between early (p4) and late (p161) passages of 17 LCL strains. Microarray data analysis showed that transcript sequences of 16 genes were differentially expressed (> 2-fold changes) in at least 16 out of 17 LCL strains. CD38 downregulation and PTPN13 upregulation were represented in all 17 LCL strains at the late passage (p161), compared to the early passage (p4). These differentially expressed genes (DEGs) included lymphocyte growth and apoptosis related genes. When LCLs proliferate up to a passage number of 160, the LCL is considered as terminally immortalized. Thus, this result suggests that the completion of LCL immortalization may require appropriate expression changes of this cellular immortalization-related gene signature.

Genomewide Association Study for Susceptibility Genes Contributing to Familial Parkinson Disease. *N. Pankratz[1], J. B. Wilk[2], J. C. Latourelle[2], A. L. DeStefano[2], C. A. Halter[1], E. W. Pugh[3], K. F. Doheny[3], J. F. Gusella[4], W. C. Nichols[5], T. Foroud[1], R. H. Myers[2], and the PSG-PROGENI and GenePD Investigators, Coordinators and Molecular Genetic Laboratories* 1) Indiana University, Indianapolis, IN; 2) Boston University, Boston, MA; 3) Johns Hopkins University, Baltimore, MD; 4) Massachusetts General Hospital, Boston, MA; 5) Cincinnati Childrens Hospital, Cincinnati, OH.

Parkinson disease (PD) is the second most common neurodegenerative disorder. Five genes have been identified with mutations that result in autosomal dominant or autosomal recessive forms of PD; however, these mutations have been found in fewer than 5% of patients, suggesting that additional genes contribute to disease risk. Recent genomewide association studies have utilized primarily sporadic PD. In contrast, we have performed the first genomewide association study in familial PD. Genotyping was performed with the Illumina HumanCNV370Duo array in 857 familial PD cases and 867 controls. A logistic model was employed to test for association under additive and recessive modes of inheritance after adjusting for gender and age. The strongest association result was with SNPs in the GAK/DGKQ region on chromosome 4 (additive model: $p = 3.4 \times 10^{-6}$; OR=1.69). Consistent evidence of association was also observed to SNCA (additive model: $p = 5.5 \times 10^{-5}$; OR= 1.35) and the chromosomal region containing MAPT (recessive model: $p = 2.0 \times 10^{-5}$; OR=0.56; additive model: p = 7.8 x 10-5; OR=0.75). Both of these genes have been implicated previously in PD susceptibility; however, neither was identified in previous GWAS studies of PD. Meta analysis was performed using data from a previous case control genomewide association study, and p-values improved for several regions, including GAK/DGKQ (additive model: $p = 2.5 \times 10^{-7}$) and the MAPT region (recessive model: $p = 9.8 \times 10^{-6}$; additive model: $p = 4.8 \times 10^{-5}$). These data suggest the identification of new PD susceptibility genes, particularly in the GAK/DGKQ region, and also provide further support for the role of SNCA and MAPT in PD susceptibility.

New linkage analysis by the Autism Genome Project (AGP) reveals strong evidence of linkage to multiple loci as well as gene-gene interactions. *V. Vieland, Autism Genetics Cooperative and Autism Genome Project* Battelle Ctr Math Medicine, Res Inst Nationwide Child Hosp, Columbus, OH.

The international AGP consortium has assembled a very large autism mutiplex family collection. The original linkage and CNV study (1181 multiplex families, 10K SNP data), published in Nature Genetics, yielded a peak ZLR = 3.6 on 11p12-p13 in the whole data set; no other loci met criteria for suggestive linkage. Here we use a quasi-Bayesian framework to reanalyze the data, sequentially updating linkage evidence across data collection sites, separately for low IQ (LIQ) and normal IQ (NIQ) families, which might differ genetically. We find strong evidence of linkage to one locus in NIQ, posterior probability of linkage (PPL)=65% on 11p15.4-15.3; as well as PPL=24% over the original 11p12-p13 peak and 22% at 11q14.1. Overall 98% of the genome shows PPL < 5%, and 90% of the genome gives evidence against linkage (PPL<2%). In the LIQ group we obtain a similarly clean plot, with several striking linkage peaks: PPL=55% on 1q31.3, 25% on 2p25.1, 50% on 11p15.2; 32% on 11p12p-13 (the original peak), 46% on 13q22.1 (previously implicated AD/specific language impairment locus), and 95% on 16q21. Other than the original peak on 11p12-13, there is no overlap in findings between LIQ and NIQ groups. Small positive evidence (PPL = 3%-17%) is also seen over CNTNAP2, the 16 microdeletion region, CENTG2, the Prader-Willi Angelman region on 15q, and the MET region on 7, all of which have been implicated in AD. In preliminary analyses using a subset of the data, we incorporated genotypes for an associated SNP in the engrailed 2 (EN2) gene and repeated the genome scan allowing for two-locus (2L) epistasis. We find evidence of interaction with EN2 at the 15q del/dup region (2LPPL=25%) in NIQ; and strong evidence at the 11p14, p15 regions (69%, 46%) and at 13q22 (54%) in LIQ, as well as at several additional loci not detected in the single-locus scan. Molecular follow up is underway, as are additional analyses involving sex differences and interactions with other candidate genes. In summary, we have obtained multiple strong linkage and epistasis signals in the largest AD linkage study to date.

Manipulation of Salience of Family History of Coronary Heart Disease: Does it Affect Intention to Screen? *C. D. Nichols[1], R. N. Rimal[2], H. P. Levy[3], E. K. Reed[3], J. A. Scott[4]* 1) Medical Genetics, Hospital of the University of Pennsylvania, Philadelphia, PA; 2) Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; 3) McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins, Baltimore, MD; 4) Genetics and Public Policy Center, Johns Hopkins, Washington, D.C.

Family history has been suggested as an accessible and valuable public health tool for promoting preventive behavior change by increasing awareness of risk for common, complex diseases like coronary heart disease (CHD). The analytic and clinical validity of family history for CHD risk assessment is established, but its efficacy in promoting behavior change has not been well studied. We manipulated the salience of family history as a risk factor to determine its effect on screening intentions. A general population sample was queried about intentions for blood cholesterol screening and beliefs that may influence the likelihood of taking preventive action based on the Health Belief Model. To study the role of family history salience, subjects were randomly assigned to read information about family history as a CHD risk factor and answer questions about their personal family history of CHD either before (n=96) or after (n=90) completing the study measures. Results: Regardless of salience manipulation, individuals with a positive family history (n=89) had higher levels of perceived susceptibility ($t=5.92$, $p<0.001$), perceived severity ($t=2.07$, $p<0.04$) and heart disease worry ($t=3.91$, $p<0.001$), compared to those with a negative family history (n=93). Perceived susceptibility and heart disease worry were associated with higher intention to screen. Multivariate regression ($R^2=0.39$) showed that important factors in intention for cholesterol screening included age, perceived barriers to screening, self-efficacy for screening, past screening behaviors, and heart disease worry. Conclusion: Individuals appear to have an exisiting understanding of family history as a risk factor for CHD. However, factors other than having a positive family history may be more significant in determining intention to get cholesterol screening.

Prolyl 3-Hydroxylase 1 and CRTAP are Mutually Stabilizing in the Endoplasmic Reticulum Collagen Prolyl 3-Hydroxylation Complex. *W. Chang, A. M. Barnes, W. A. Cabral, J. C. Marini* BEMB, NICHD, NIH, Bethesda, MD.

We recently identified null mutations in cartilage associated protein (CRTAP) and prolyl 3-hydroxylase 1 (P3H1/LEPRE1) as the cause of two novel recessive forms of OI, Types VII and VIII, respectively. CRTAP and P3H1, along with cyclophilin B, form a complex in the endoplasmic reticulum (ER) which 3-hydroxylates the Pro986 residue of 1(I) and 1(II) collagen chains. We used cultured dermal fibroblasts from type VII and VIII OI probands to investigate the interaction of CRTAP and P3H1. P3H1, which contains the enzymatic activity of the complex, is absent in CRTAP-null fibroblast lysates and, conversely, CRTAP is minimally detectable in P3H1-null lysates. Immunofluoresence microscopy confirmed reduced CRTAP levels in P3H1-null cells and support ER localization of CRTAP. However, P3H1 or CRTAP mRNA levels, respectively, were normal in cells in which null mutations in the other gene caused reduced transcript levels due to NMD. Moreover, CRTAP and P3H1 are stable in normal fibroblasts after translation inhibition with cyclohexmide. These data imply that P3H1 and CRTAP are normally mutually stabilizing. In contrast, cyclophilin B was as abundant in both sets of mutant cell lines as in control. Stable transfection of a full-length CRTAP expression plasmid into CRTAP null fibroblasts restored both CRTAP and P3H1 protein levels, and reduced type I collagen overmodification. A series of CRTAP deletion constructs was also transfected to explore crucial interaction domains. CRTAP was not restored in P3H1-null fibroblasts treated with the proteosomal inhibitor MG132; instead, the increased CRTAP in media may reflect absence of a CRTAP ER retention signal. P3H1 is detectable in lysates of CRTAP-null cells treated with MG132. However, some P3H1 apparently occurs in insoluble pellets from CRTAP-null cell lysates, suggesting P3H1 aggregates in the absence of the complex. The mutual stabilization of P3H1 and CRTAP in the ER collagen prolyl 3-hydroxylation complex may be the underlying mechanism for the similar characteristics of types VII and type VIII OI.

Searching for nucleotide changes in miRNAs and their target genes in circadian clock modulators for mood disorders. *E. Saus[1], V. Soria[2], F. Vivarelli[1], J. M. Crespo[2,3], J. M. Menchón[2,3], M. Urretavizcaya[2,3], X. Estivill[1,4], M. Gratacòs[1]* 1) CIBERESP (CIBER en Epidemiología y Salud Pública), Genes and Disease Program, Center for Genomic Regulation (CRG), Barcelona; 2) Mood Disorders Clinical and Research Unit, CIBER-SAM, Psychiatry Department, Bellvitge University Hospital, LHospitalet de Llobregat; 3) Department of Clinical Sciences, Bellvitge Campus, Barcelona University, Barcelona; 4) Experimental and Health Sciences Department, Pompeu Fabra University, Barcelona, Catalonia, Spain.

It is widely supported that the pathophysiology of mood disorders (MD) is influenced by circadian rhythms. Recently, two independent studies have reported five different miRNAs (hsa-miR-219-1/132/183/96/182) as modulators of the endogenous circadian clock located in the suprachiasmatic nucleus in mice, and have shown experimental evidence for some of the genes involved in the molecular clock machinery as targets sites. Thus, we hypothesized that variations in the miRNAs sequences involved in circadian rhythms or in their target sites could be related to a higher susceptibility to MD. Once the five miRNAs with their respective targets sites were confirmed in humans, we re-sequenced them in a sample of 365 MD patients and 342 control individuals. When analyzing 360 MD patients, no variation was found in hsa-miR-132, hsa-miR-219-1, or their respective target sites in RFX4 and PHLPP genes or hsa-miR-183. Two changes were found in the precursor form of pre-miR-96, one present in MD patients and in controls and the other only found in one MD patient. Regarding hsa-miR-132, changes were also found in the precursor form: two changes found each in one MD patient, one new change found in one MD patient as well as in two control subjects, and one variation found in a high frequency in both MD and control individuals. This is the first time that the circadian clock regulation by miRNAs is explored in MD patients, which could represent a first step to elucidate its underlying mechanisms.

Genetic polymorphisms of the insulin receptor substrate-1 (IRS1) gene and profiles of clopidogrel-induced antiplatelet effects in type 2 diabetes mellitus patients with coronary artery disease. *M. Zanoni[1], P. Prandini[1], D. J. Angiolillo[2], E. Bernardo[3], A. Fernandez-Ortiz[3], C. Macaya[3], T. A. Bass[2], E. Trabetti[1], P. F. Pignatti[1]* 1) Department of Mother and Child, University of Verona, Verona, VR, Italy; 2) Division of Cardiology, University of Florida College of Medicine-Shands Jacksonville, Jacksonville, FL, United States; 3) Cardiovascular Institute, San Carlos University Hospital, Madrid, Spain.

Background: Patients with T2DM have reduced responsiveness to the platelet P2Y12 receptor antagonist clopidogrel compared to non-diabetics. However, variability in clopidogrel-induced antiplatelet effects has been observed among T2DM and those with elevated platelet reactivity have a higher risk of atherothrombotic events. Since variation in insulin sensitivity modulates platelet P2Y12 signaling, the aim of this study was to evaluate if SNPs of the insulin receptor substrate-1 (IRS-1) are associated with variation in the response to clopidogrel in T2DM patients. Methods: A total of 7 tagSNPs (rs1801278, rs11683087, rs1801123, rs1896832, rs956115, rs2251692, rs6725330) were determined in 180 T2DM patients with coronary artery disease in a steady state phase of clopidogrel therapy. Patients were classified into carriers and non-carriers of the variant allele for each SNP according to a dominant model. In addition, haplotype distribution of IRS-1 genes within our population was assessed. Platelet function was determined by LTA following 20 M adenosine diphosphate stimuli (ADP). Platelet aggregation according to genotype and haplotype was determined. Results: The 7 tagSNPs were accountable for 93% of the haplotype distribution of the IRS-1 gene. Platelet aggregation was 5515% in the overall population. Preliminary results showed an association between carriers of the variant C allele of the rs956115 SNP (n=34) and the highest degree of platelet reactivity (6014%; $p<0.05$). Conclusions: rs956115 polymorphism of IRS-1 gene is associated with lower clopidogrel-induced antiplatelet effects in T2DM patients with coronary artery disease.

Differential miRNA-mediated regulation of two isoforms of the receptor for neurotrophin-3 (NTRK3). *M. Guidi[1,2], B. Kagerbauer[1,2], M. Muinos-Gimeno[1,2], X. Estivill[1,2,3], Y. Espinosa-Parrilla[1,2]* 1) Genes & Disease Program, Center for Genomic Regulation (CRG), Barcelona, Spain; 2) Public Health and Epidemiology Network Biomedical Research Center (CIBERESP); 3) Pompeu Fabra University (UPF), Barcelona, Spain.

Neurotrophins are a family of key signaling molecules in the development of the nervous system. NTRK3 is the preferential receptor for neurotrophin-3 (NT-3). Several NTRK3 isoforms are known; the best studied are the full-length kinase-active form (150 kDa) and a truncated non-catalytic form (50 kDa) lacking the kinase domain. The full-length and the truncated isoform show different 3UTR regions, suggesting possible differences in their post-transcriptional regulation. Fifty-four miRNAs were tested on the two 3UTRs using a luciferase-based assay in HeLa cells; eleven miRNAs causing a significant down-regulation of the reporter gene were identified, with no overlapping between the subsets of miRNAs regulating the two isoforms. Overexpression of the eleven miRNAs were performed in SH-SY-5Y neuroblastoma cells, to validate on endogenous NTRK3 the results obtained with the luciferase constructs. Morphological changes have been observed upon overexpression of some of the miRNAs; interestingly, one of them is the brain-enriched miR-128, which is involved in neuronal differentiation. The consequences of miRNA-mediated downregulation of NTRK3 are now being evaluated analyzing downstream elements in the pathways activated by the two isoforms.

Array CGH Uncovering Cancer Predisposition Genes in Children with Syndromic Presentations: Important Tool for Genetic Counseling and Medical Management. *L. Medne, P. Goldenberg, M. J. Falk, T. H. Shaikh, E. H. Zackai* Div Hum Gen, Children's Hosp Philadelphia, Philadelphia, PA.

We present two patients with multiple congenital anomalies and developmental delays where array CGH identified novel genomic microdeletions. Further review of the genes in the deleted regions revealed haploinsufficiency for a tumor suppressor gene in both cases leading to cancer predisposition syndromes and need for appropriate surveillance. Patient 1 presented at 20 months for evaluation of hypotonia, severe, global delays, non-specific dysmorphic brain anomalies, dysphagia, GERD, unexplained immunodeficiency requiring monthly IVIG treatments, dermoid cysts, retinal hamartomatous lesion, iris hypoplasia, strabismus, and renal and ovarian cysts. Extensive prior genetic and metabolic work-up was normal. Given the multi-system involvement, oligonucleotide-based array analysis was pursued. While the 100K array was normal, 550K SNP array identified a de novo ~900 Kb deletion at 17p13.1 encompassing the *TP53* tumor suppressor gene. Germline mutations in *TP53* are known to cause Li-Fraumeni syndrome. Patient 2 presented in the newborn period for evaluation of central apnea, feeding and swallowing difficulties, ventricular septal defect, microcephaly, simplified gyral pattern, under-opercularizaton, bilateral camptodactyly, abnormal neurologic examination, and dysmorphia. Clinical BAC-based CGH analysis revealed 9.3 Mb deletion at 1p36.13-1p36.22 encompassing ~60 genes. The deletion includes the *SDHB* gene with germline mutations causing familial paraganglioma and pheochromocytoma with age-dependent penetrance. Her deletion does not include *NBL1,* which predisposes to neuroblastoma. The deletion includes *MFM2* and *KIF1B* genes, thus predisposing to Charcot-Marie-Tooth, type 2A2 and type 2A1 respectively. Interpretation of copy number variations on the arrays can be challenging as the findings are often novel, but it is of the utmost importance to carefully review all the genes in the deleted region. In both cases, cancer predisposition syndrome identification was unexpected based on the presenting features but significantly altered genetic counseling and patient management.

Mutations of *AFG3L2* gene (SCA28) in autosomal dominant cerebellar ataxias. *C. Cagnoli[1], G. Stevanin[2], A. Durr[2,3], P. Ribai[2], S. Forlani[2], A. Brussino[1], P. Pappi[1], L. Pugliese[4], M. Barberis[1], R. L. Margolis[5], S. E. Holmes[5], S. Padovan[1,6], N. Migone[1], D. Di Bella[7], F. Taroni[7], A. Brice[2,3], A. Brusco[1]* 1) Dept Genetics, Biol & Biochem, Univ Torino, Torino, TO, Italy; 2) INSERM/UPMC UMR_S679, Paris, France; 3) Dept. Genetics and Cytogenetics, Pitie-Salpetriere Hosp., Paris, France; 4) SAFAN, Bioinformatics Turin; 5) Dept. Psychiatry and Behavioral Sciences, The Johns Hopkins University, Baltimore, MD, USA; 6) CNR Torino; 7) Genetics of Neurod. & Metabolic Diseases, Fondazione IRCCS, Ist. Neurologico C.Besta, Milan, Italy.

Spinocerebellar ataxias (SCA) are a genetically heterogeneous group of autosomal dominant neurodegenerative disorders. At present, 26 loci and 16 genes are known. Recently, we have identified *AFG3L2*, encoding a mitochondrial protein, as the causative gene for SCA28 (18p11-q11)(DiBella et al.,ASHG 2008). We have screened the 17 coding exons of *AFG3L2* in a group of 376 unrelated adult patients with autosomal dominant cerebellar ataxia (ADCA). Patients were Caucasian, negative for triplet-expanded SCA1-3, 6, 7 genes. Using DHPLC followed by direct sequencing, we found six different missense mutations (T654I, M666R, M666T, M666V, G671R and G671E) in eight patients (8/376, 2.1%). T654I and M666V mutations were present in two different families that shared a common haplotype. All mutations clustered in exons 15-16, corresponding to the peptidase M41 domain. The mutations hit evolutionary conserved aminoacids. Five changes are predicted to destabilize the protein folding by bioinformatics analysis. None of these mutations was found in a survey of 200 chromosomes from healthy controls. Age at onset was 6-60 (mean 27yr) in 18 affected patients from the mutated families. Examination of affected individuals revealed universal gait and limb ataxia (18/18, 100%) and frequent lower limb hyperreflexia (11/17, 64%), nystagmus (11/16, 69%), and ophtalmoplegia (10/17, 59%). In conclusion, SCA28 seems a rare ADCA (>1%), caused by mutations grouped in the carboxy-terminal region of AFG3L2 protein. The clustering and the missense nature of the mutations are not indicative of haploinsufficiency as pathogenetic mechanism.

The relative effectiveness and efficiency of genomic test-based algorithms in the identification of Lynch syndrome index cases among unselected Colorectal cancer population. *M. Williams[1], R. Burt[2], J. Williams[3], W. Samowitz[4], J. Gudgeon[5]* 1) Dir, IHC Clinical Genetic Inst, LDS Hosp, Salt Lake City, UT; 2) Dir, Huntsman Cancer Institute, University of Utah, Salt lake City, UT; 3) Intermountain Healthcare Oncology Genetic Services Salt Lake City, UT; 4) Associated Regional and University Pathologists, Salt Lake City, UT; 5) IHC Clinical Genetic Inst, LDS Hosp, Salt Lake City, UT.

Statement of Purpose: Identifying patients with Lynch syndrome (LS) using clinical data, family history and tumor histology (for those presenting with colorectal cancer (CRC)) has been hampered by relatively poor predictive value. Recently, several published papers have explored models using molecular analysis of CRC specimens to identify patients with LS. The purpose of this study is to use decision analysis to explore the viability, effectiveness, and costs of different testing algorithms in anticipation of implementing this in an integrated healthcare system. Methods: Decision analytic models were constructed to estimate the diagnostic performance of different molecular screening pathways for unselected CRC patients to detect those at risk for LS. Screening tests considered were immunochemistry (IHC), microsatellite instability (MSI), and *BRAF*. Additional tests modeled in reflex cascades were gene sequencing and deletion (duplication, rearrangement) testing of the four mismatch repair genes involved in LS. Outcomes were limited to test results: true and false positive and -negative. The proportions of these parameters were then used to calculate sensitivity, specificity, positive and negative predictive values, and efficiency of the different test algorithms. Test costs were based on charges from the reference lab. Summary of Results: Modeling demonstrates that screening with IHC without incorporation of BRAF or MSI testing maximizes identification of LS index cases as well as minimizing costs compared to other screening algorithms that start with MSI or *BRAF* or that start with IHC and include *BRAF* or MSI. Conclusions: IHC of unselected CRC tumors optimizes detection of patients with LS while minimizing cost of the screening program.

Prognosis and treatment based upon genetic subtypes of Hermansky-Pudlak syndrome. *M. Huizing[1], R. Hess[1], A. Helip-Wooley[1], R. Fischer[1], K. O'Brien[2], G. Golas[2], WA. Gahl[1,2]* 1) MGB, NHGRI, NIH; 2) Office of Rare Diseases, OD, NIH, Bethesda, MD.

Hermansky-Pudlak syndrome (HPS) comprises a group of autosomal recessive disorders of lysosome-related organelle (LRO) biogenesis. Eight human genes (*HPS1-HPS8*) are associated with eight clinical subtypes. Oculocutaneous albinism, due to abnormal melanosome formation, and prolonged bleeding, due to absent platelet dense bodies, occur in all HPS subtypes. Sporadic features include a fatal pulmonary fibrosis, granulomatous colitis and neutropenia. Over the past 10 years we extensively studied and subtyped 232 HPS patients. HPS-1 (166 patients, 23 mutations) constitutes the largest group due to a founder mutation in NW Puerto Rico. HPS-2 (3 patients, 4 mutations) results from mutations in *AP3B1*, coding for a subunit of adaptor complex-3, a coat protein that mediates vesicle formation. Our study of HPS-3 yielded 29 patients (19 mutations), with *HPS3* founder mutations in central Puerto Rico and in Ashkenazi Jews. We also identified 12 HPS-4 patients (10 mutations), 6 HPS-5 patients (8 mutations) and 4 HPS-6 patients (7 mutations). Our nearly 100 non-Puerto Rican patients are more than the total number reported elsewhere in the world. Our clinical characterization of these patients allowed us to draw several critical conclusions. First, only HPS-1 and HPS-4 patients develop pulmonary fibrosis. This knowledge allows us to enter appropriate patients into our clinical trial of pirfenidone, an antifibrotic agent previously shown to slow the decline in pulmonary function in HPS-1 patients. Second, compared with HPS-1 and HPS-4 patients, HPS-3, HPS-5 and HPS-6 patients are clinically milder and have no pulmonary involvement. Third, HPS-2 patients are the only subtype prone to infections, and their neutropenia is G-CSF responsive. Fourth, our 12 unclassified HPS patients provide opportunities to identify new HPS-causing genes. These studies provided important insights into genotype-phenotype correlations. An accurate subtype diagnosis now carries important prognostic and therapeutic implications, and future studies can provide insights into the cell biology of LROs.

Consanguineous matings, partial selective sweeps, and population-specific founder effects detected from patterns of homozygous tracts. *A. R. Boyko, A. Auton, A. R. Indap, K. E. Lohmueller, A. G. Clark, C. D. Bustamante* Cornell University, Ithaca, NY.

The degree to which deleterious recessive mutations contribute to disease is largely dependent on the rate of autozygosity or identity by descent. Runs of homozygosity (ROHs) are indicative of autozygosity and can be easily detected with dense SNP genotyping arrays. Here, we develop a novel hidden Markov model to detect ROHs and apply it to a global panel of 4000 individuals genotyped on the Affymetrix 500K SNP array. This panel includes European and Asian populations that have undergone founding bottlenecks, Latin American populations founded through recent admixture, and Middle Eastern populations with less severe demographic histories and relatively high levels of genetic diversity. Our HMM is more robust to SNP ascertainment biases, variation in SNP density, and population substructure than the inbreeding statistic (F) or previous sliding window approaches for ROH detection. All populations contain individuals with long ROHs (>2cM) indicative of consanguinity, although the average individual from each population contains few if any such tracts. In contrast, shorter ROHs (<2cM) are found in all individuals and the cumulative length of these tracts differs significantly between populations. The location and distribution of shorter ROHs correspond to regions of low population-wide haplotype diversity often caused by founder effects or partial selective sweeps. Long ROHs, although rarer and distributed more or less at random across the genome, account for more than 20% of the genome in some individuals and contribute 10-25% of the autozygous SNPs found in a population. The proportion of highly autozygous individuals differs significantly between populations largely due to marriage customs. Simulations show a good correspondence between the observed and expected proportion of the genome that is in ROH. Surprisingly, the rate of purging of deleterious recessive alleles inferred from the distribution of ROHs is only marginally faster than the rate in a randomly mating population, suggesting that such alleles account for an appreciable fraction of human genetic disease risk.

Recurrent reciprocal deletions and duplications within 1q21.1: risk factors for developmental abnormalities with variable expressivity and incomplete penetrance. *N. Brunetti-Pierri, J. Berg, F. Scaglia, C. Bacino, T. Sahoo, S. Lalani, P. Stankiewicz, S. W. Cheung, A. Patel, Referring phisicians* Dept Molec & Human Genetics, Baylor Col Medicine, Houston, TX.

The chromosome 1q21.1 is a highly complex region containing large low-copy repeats (LCRs). Microdeletions within the distal 1q21.1 region have been associated previously with congenital heart defects and mental retardation, while reciprocal microduplication of the same region has also been reported in a patient with dysmorphic features and mental retardation. Recently, a distinct, more proximal region within 1q21.1 was implicated in Thrombocytopenia-Absent Radius (TAR) syndrome. We have identified 21 probands with 1q21.1 microdeletion and 13 probands with 1q21.1 microduplication using targeted array comparative genomic hybridization (array-CGH) in a population referred to for a variety of clinical indications. These copy-number variants (CNVs) clustered into groups involving only the distal 1q21.1 region or both distal 1q21.1 and TAR regions. The CNVs were inherited in the majority of cases in which parental studies were available, leading to difficulty in determining causation of the associated clinical phenotypes. The most consistent features appear to be microcephaly in microdeletion cases and a trend toward macrocephaly in microduplication cases. In our experience, the phenotypes observed in individuals with 1q21.1 CNVs are highly variable and cardiovascular malformations are uncommon. Familial CNVs are frequently observed in array-CGH studies and often raise concerns regarding incomplete penetrance for some CNVs. We cannot rule out that the phenotypes observed in our probands result from the combination of 1q21.1 microdeletion or microduplication and some as-yet unidentified recessive alleles or other CNVs inherited from the two parents. However, our findings suggest that CNV within the 1q21.1 region may predispose or contribute to developmental abnormalities, albeit with variable expressivity and incomplete penetrance.

When a case is not a case: effects of phenotype misclassification on the power of the transmission disequilibrium test for affected child trios. *S. Buyske[1, 2], G. Yang[2], T. C. Matise[2], D. Gordon[2]* 1) Statistics and Biostatistics Dept, Rutgers Univ, Piscataway, NJ; 2) Genetics Dept, Rutgers Univ, Piscataway, NJ.

Phenotype misclassification in genetic studies can decrease the power to detect association between a disease locus and a marker locus. Many studies have looked at genotyping errors, but phenotype misclassification can have far greater effects. Studies of phenotype misclassification to date have focused on case-control designs. We examine the effect of phenotype misclassification on the transmission disequilibrium test (TDT) applied to complete case-parent trios.

We study misclassification by computing the effect on the non-centrality parameter associated with the TDT statistic where there is linkage and association of a marker locus with a disease locus. Simulations verify our analytic work.

To maintain equivalent power under phenotype misclassification, the sample size must be increased over the no-error size. The required increase grows as the disease prevalence decreases and as the misclassfication rate increases. A misclassification rate of 5% in a disease with prevalence 1% can require almost 40 times the sample size for equivalent power of a study with zero misclassification.

Given the potentially substantial power loss for the TDT in the presence of misclassification, we recommend that researchers incorporate power loss due tophenotype misclassification into their study design for genetic association using trio data. We have developed freely available software that computes power loss for a fixed sample size or sample size for a fixed power in the presence of phenotype misclassification.

Sequence variants analysis of celiac disease candidate genes TAGAP and VIL2. *A. Szperl[1], G. Trynka[1], A. Zhernakova[2], C. Wijmenga[1]* 1) Genetics Department, UMC Groningen, The Netherlands; 2) Complex Genetics Section, DBG-Dept Medical Genetics, UMC Utrecht, The Netherlands.

Celiac disease (CD) is the most common food intolerance in Western populations; it is caused by a strong immune response to gluten. The HLA-DQ2/DQ8 locus explains 40% of the heritable risk. To identify additional CD genes, we have performed both whole genome linkage analysis and genome-wide association studies (GWAS). One of the linkage peaks in a large CD family mapped to chromosome 6q25.3, with the strongest signal at marker D6S969, located ~175 kb upstream of the T-cell activation Rho-GTPase activating protein (TAGAP) and ~96 kb downstream of the villin-2 gene (VIL2). We also identified TAGAP as a CD susceptibility gene in our GWAS. TAGAP is highly expressed in bone marrow, spleen, and thymus. It belongs to the RhoGAP family of proteins, which are known to be key regulators that link signaling pathways with the organization of cytoskeleton in the cell. Our group recently identified the myosin IXb (MYO9B) gene, which is also a Rho-GTPase activating protein (RhoGAP), as a risk factor for CD in a Dutch population. Since our two independent studies pointed to the TAGAP gene, we investigated TAGAP for putative mutations by sequence analysis in ten members of the large CD family (7 patients and 3 unaffected as controls). We sequenced 12 kb of genomic DNA, including the coding part, promoter and conserved regions, but found only two sequence variants in one of the controls. Since we cannot exclude a duplication or deletion in this CD locus, we are now investigating it. In addition to TAGAP, VIL2 could also be a perfect CD disease gene; it is highly expressed in the epithelial cells in the small intestine where it functions as a connection between the plasma membrane and actin cytoskeleton. Using the same strategy as for TAGAP, we sequenced VIL2 and identified 25 sequencing variants. Two variants segregated with the disease phenotype in the linkage region but were absent in the controls. Interestingly, one is located in the predicted Vil2 promoter region and the second may be involved in splicing. We are studying both variants further.

Structural insights on molecular pathogenesis of novel ornithine carbamoyl transferase (OTC) mutations in OTC deficiency. *S. GOBIN-LIMBALLE[1], M. MAGEN[1], V. SERRE[2], P. DE LONLAY[3], R. GESNY[1], V. VALAYANNOPOULOS[3], J. BENGOA[1], D. RABIER[4], N. GIGAREL[1], A. MUNNICH[1], J. STEFFANN[1], J. P. BONNEFONT[1]* 1) Genetics Dpt,; 2) INSERM Unit U781 and Université Paris Diderot; 3) Pediatrics Dpt; 4) Biochemistry Dpt, hopital Necker, Paris, France.

Ornithine Transcarbamylase (OTC) deficiency is the most common inherited defect of the urea cycle. This X-linked semi-dominant disease manifests through distinct phenotypes, considered as more severe in male vs female. The neonatal form usually occurs in males, as life-threatening hyperammonemia episodes, while the late-onset form variably presents as Reye-like attacks, acute liver failure, or psychomotor retardation. While over 250 OTC mutations have been reported, the genotype-phenotype relationships remain unclear. We detected novel OTC mutations in 18 OTC-deficient families, being missense ones (11), frameshift mutations (5) or large deletions (2). We analyzed the missense mutations, using a 3-D structural model of the human dodecamer enzyme, based on alignment of the human monomer and the P. furiousus dodecamer crystal structures. These mutations were predicted to impact the catalytic domain (6/10), or structural domains implied in the protein stability (2/10) or in putative interaction with other proteins (2/10). They predominantly involved proline residues, suggested to result in alpha-helixes destabilization. We matched molecular-, in silico-, and clinical data. Truncating mutations/deletions predominantly resulted in a neonatal form (7/9) in females (3) and males as well (4). Irrespective of their predicted structural/functional impact, missense mutations i) were as serious as truncating mutations (death in 9/11 and 3/7 families with missense and truncating mutation/deletions, respectively); ii) resulted either in neonatal presentation, predominantly in males (6/7), or in delayed form, irrespective of the patient gender (4 males/5 females). Comparison with data of literature indicated that different substitutions affecting the same amino acid may result in various clinical presentations. We are carrying a large-scale in silico structural analysis of hitherto reported missense mutations to assess potential relationships between predicted impact and the resulting phenotype.

Association of BRCA1 founder mutations prevalent in Latvia with haplotype tagged SNPs. *L. Tihomirova, L. Pliss, S. Skudra* Latvian Biomedical Research and Study Centre, Riga.

It has been shown that the BRCA1 mutation 4154delA detected in unrelated individuals from Latvia, Poland and Russia is a founder mutation with common ancestral origin (Skudra et al Familial cancer, in press) We have compared the age at onset of disease in Latvian breast and ovarian cancer patients carrying the 4154delA or 5382insC mutation (the two most prevalent mutations in Latvia). Regardless the numbers of mutation carriers are not large (40 and 60 respectively, altogether detected) we have found similar, 5 year difference in the age at onset of disease between breast as well as between ovarian cancer patients ($p=0.01$). The further analysis of genetic variation in the BRCA1 region revealed several haplotype tagged SNPs specific for both mutation carriers. The association of the 185delAG mutation with the genetic variant in the NBR2 gene, not detected in carriers of 4154delA or 5382insC, was found as well. The analysis of specific, haplotype tagged genetic variants are useful for epidemiologic studies and possible finding new susceptibility loci associated with breast cancer.

Knockout Models in Mice and Men Suggest a Proatherogenic Role for USF1. *P. P. Laurila[1], K. J. Merikanto[1], J. Perttilä[1], J. Saharinen[1], M. Gentile[1], J. Naukkarinen[1], P. K. Laurila[2], A. Tuomainen[3], C. Ehnholm[1], V. M. Olkkonen[1], M. Jauhiainen[1], L. Peltonen[1,4]* 1) Institute of Molecular Medicine, NPHI, Helsinki, Finland; 2) Helsinki Univ Central Hospital, Helsinki, Finland; 3) Institute of Dentistry, Univ of Helsinki, Finland; 4) The Wellcome Trust Sanger Institute, Cambridge, UK.

Disturbances in body lipid homeostasis are tightly linked with cardiovascular disease. We recently established the association of USF1 transcription factor with familial combined hyperlipidemia, a common dyslipidemia characterized by high blood triglycerides (TG) and cholesterol in Finnish families. We have generated a strain of Usf1 knockout (-/-) mice which along with their +/- and +/+ littermates (n=12) were fed with Western diet rich in TG and cholesterol for 8 weeks. After the diet Usf1 -/- mice had significantly lower blood ($p<0.05$) and VLDL ($p<0.01$) TG than their +/+ littermates. By using Affymetrix expression arrays, we observed that lipoprotein lipase (Lpl) and ApoA5 mRNA levels were elevated ($p<0.01$) in -/- mice liver and muscle as compared to controls, consistent with the TG phenotype. In -/- mice adipose tissue, the cholesterol biosynthetic pathway was significantly down-regulated when compared to their +/+ littermates. We also established a cellular model representing USF1 knock-down, transfecting human hepatoma cells (HuH7) with USF1 targeting siRNAs. We incubated the cells for 30 min in [3H]acetic acid, followed by a chase, and quantified the [3H]cholesterol and TG synthesized. A decreased amount of both [3H]cholesterol and [3H]TG ($p<0.05$) was observed in siRNA treated cells as compared to controls. Again, with Affymetrix microarrays we observed that several cholesterol biosynthetic pathway enzymes were down-regulated in USF1 knockdown cells in addition to up-regulation of a number of apolipoprotein, many of which are thought to be antiatherogenic by nature. Since the loss-of-function of USF1 caused a decrease in plasma TG in vivo as well as cholesterol and TG biosynthesis in vitro, we hypothesize that USF1 might be proatherogenic and its knockdown in appropriate tissues actually has a favorable cardiovascular effect.

Genetic Background of Severe Obesity among Finns. *K. J. Merikanto[1], J. Naukkarinen[1], K. Silander[1], T. Tanninen[1], K. Pietiläinen[2], V. Salomaa[3], K. Kontula[4], P. Mustajoki[4], A. Rissanen[2], J. Kaprio[5], L. Peltonen[1,6]* 1) Institute of Mol. Medicine , National Public Health Institute, Helsinki; 2) Obesity Research Unit, Dept. of Psychiatry, Helsinki Univ Central Hospital; 3) Dept of Epidemiology and Health Promotion, National Public Health Inst, Finland; 4) Dept of Med, Helsinki Univ Central Hospital, Helsinki, Finland; 5) Finnish Twin Cohort Study, Dept. of Public Health, Univ of Helsinki; 6) The Wellcome Trust Sanger Institute, Cambridge, UK.

The increase in obesity in the Western world poses an enormous challenge to public health. In addition to sedentary lifestyle, evidence of the genetic component of obesity is now accumulating. We selected six previously well established obesity and dyslipidemia associating genes (PPAR, APOB, CDH2, GCKR, FTO and INSIG2) as well as a frequently replicated CHD locus on chromosome 9p21 to test whether variants in these regions were associated with obesity (BMI>30). 54 haplotype-tagging SNPs were genotyped in our case-control material. The cases (n=421, BMI>30) selected for genotyping were either obese individuals of Finrisk97 (FR97) population cohort or severely overweight patients from the obesity clinic at the Helsinki Univ Hospital. The controls (n=439, BMI <25) were selected from the lean FR97 subjects. Associations were obtained for PPAR (p=0.009), APOB (p=6x10-5), FTO (p=5x10-5) and for 5 SNPs in the 9p21 locus. The association signals at 9p21 were followed up by evaluating the expression levels of nearby transcripts in fat biopsies of a collection of MZ twin pairs most discordant for BMI (n=14 pairs, 15.2 kg mean weight difference). We identified the CDKN2B gene in the 9p21 locus as expressed significantly higher in the obese twin fat tissue (1.6-fold change, p=0.0003), suggesting that CDKN2B may be a functionally relevant obesity gene in this locus. Conclusion: We were able to replicate association with obesity in 3 out of 6 previously identified genes, and by combining SNP association data with global transcript profiles in fat, identify a potentially novel obesity associated gene immediately adjacent to the 9p21 locus previously associated with CHD.

Gene x Gene Interaction at the allelic level using a generalize linear model. *J. Jung* Medical & Molecular genetics, Indiana Univ Sch Medicine, Indianapolis, IN.

It has been widely recognized that genexgene interaction is likely to have an important role in the risk of complex disease. We propose a statistical approach that can detect genexgene interactions at the allelic level contributing to a variety of traits related with disease based on generalized linear regression. Our proposed method assigns a score to each unrelated subject according to their allelic combination inferred from the observed genotypes at two or more unlinked SNPs, and then tests for association of the allelic score at the all possible allelic combinations with a dependent trait. By testing for the association of allelic combinations at multiple unlinked loci, the interaction can be assessed both in cases where the SNP allelic association can and cannot be detected as a main effect. Based on simulation study, we investigate the analytical properties of the proposed methods in terms of type I error rate and power and demonstrate that the allelic approach achieves greater power than the more commonly used genotypic approach to test for gene x gene interaction.

Role of the C1236T (rs1128503) polymorphism of the MDR-1 Gene on Clopidogrel Responsiveness. *P. Prandini[1], M. Zanoni[1], D. J. Angiolillo[2], E. Bernardo[3], A. Fernandez-Ortiz[3], C. Macaya[3], T. A. Bass[2], E. Trabetti[1], P. F. Pignatti[1]*
1) Dept of Mother and Child, University of Verona, Verona, Verona, Italy; 2) Division of Cardiology, University of Florida College of Medicine-Shands Jacksonville, Jacksonville, FL, United States; 3) Cardiovascular Institute, San Carlos University Hospital, Madrid, Spain.

Background: Clopidogrel intestinal absorption and active metabolite formation are influenced by P- glycoprotein-mediated efflux. The functional activity of P-glycoprotein is under genetic control by the Multi Drug Resistance-1 (MDR-1) gene. If genetic variations of MDR-1 contribute to variability in clopidogrel response in patients with coronary artery disease remains poorly explored. Methods: The C1236T (rs1128503) polymorphism of the MDR-1 gene was assessed in 62 patients. Patients were divided into 2 groups: carriers and non-carriers of the T allele. Platelet aggregation was performed before and 24 hours after clopidogrel administration. Standard (300mg; n=45) and high (600mg: n=17) loading dose regimens were used. All patients were on treatment with aspirin (100mg/day). Peak platelet aggregation was assessed by LTA using 6 mol/L ADP stimuli. Post-treatment platelet reactivity and percentage inhibition of platelet aggregation (IPA) were determined. Results: 71% and 29% of the study population were T and non-T allele carriers, respectively. At baseline, there were no differences in platelet aggregation between the two groups. At 24 hours there were no differences in post-treatment platelet reactivity between groups following a 300mg loading dose administration. However, following a 600mg loading dose administration, post-treatment platelet reactivity was significantly higher in T allele carriers (3511% vs 163%; p=0.006). Accordingly, there were no differences in IPA following a 300mg dose and IPA was significantly lower in T allele carriers following a 600mg dose (4418% vs 735%, p=0.001). Conclusions: The C1236T polymorphism of the MDR-1 gene modulates clopidogrel responsiveness in the acute phase of treatment when using high loading dose regimens.

The Indications Leading to Termination of Pregnancy in a Referrence Hospital: Two Years Experience. *E. Ataman Arikan[1], H. Onay[1], A. Alpman[1], S. Sagol[2], O. Bilgin[2], S. Ozbek[3], O. Zekioglu[4], N. Kultursay[5], M. Akisu[5], I. Ulman[6], F. Ozkinay[1,5]* 1) Dept Medical Genetics, Ege Univ, Izmir, Turkey; 2) Dept Obstetrics and Gynecology, Ege Univ, Izmir, Turkey; 3) Dept Radiodiagnostic, Ege Univ, Izmir, Turkey; 4) Dept Pathology, Ege Univ, Izmir, Turkey; 5) Dept Pediatrics, Ege Univ, Izmir, Turkey; 6) Dept Pediatric Surgery, Ege Univ, Izmir, Turkey.

Recently, a number of major abnormalities could be able to detected prenatally in the fetus leading to termination of pregnancy (TOP). In this study, the distrubution of indications in cases undergone TOP was investigated in our medical school hospital. Between 2006-2008 years, 132 cases were referred to prenatalogy council of Ege University Medical School Hospital for TOP. They were classified into two groups, early (20 weeks) and late (20-24 weeks) gestational age. TOP are not performed after 24th week of gestation in our hospital. TOP was performed in 74 (56.1%) out of 132 cases before the 20th week of pregnancy. In both groups the most common indication was major ultrasonographic abnormalities in the fetus with a frequency of 67.4%. This was followed by chromosomal abnormalities which was detected in 12.8% of cases. Among the abnormalities detected on ultrasonographic examination central nervous system abnormalities (46.5%), urinary system abnormalities (21.5%) and skeletal abnormalities (10.2%) were the most common indications for TOP. Karyotype analysis was performed after termination in 44 fetuses having abnormal ultrasonographic findings and it was found to be normal in 34 cases (77.2%). Turner Syndrome in 2 fetuses, Trisomy 13 in fetus, Trisomy 18 in 1 fetus and Trisomy 21 in 1 fetus were detected. As a conclusion, before the 24th week of gestation, the most common indications for TOP are major ultrasonographic abnormalities and chromosomal abnormalities in the fetus.

Comprehensive genetic analysis and thorough evaluation of phenotypes according to Ghent criteria in 135 Japanese patients with suspected Marfan syndrome. *H. Morisaki[1], K. Akutsu[2], H. Ogino[3], T. Morisaki[1]* 1) Dept Bioscience, Natl Cardiovasc Ctr Res Inst, Osaka, Japan; 2) Dept Cardiovasc Med, Natl Cardiovasc Ctr, Osaka, Japan; 3) Dept Cardiovasc Surg, Natl Cardiovasc Ctr, Osaka, Japan.

Marfan syndrome (MFS) is an autosomal dominant connective tissue disorder primarily involving skeletal, ocular, and cardiovascular systems caused by mutations of *FBN1* gene. Loeys-Dietz syndrome due to mutations of *TGFBR1* or *TGFBR2* has many overlapping phenotypes with MFS. In order to evaluate the contribution of *FBN1*, *TGFBR1*, and *TGFBR2* mutations to the MFS phenotypes, we conducted a comprehensive genetic study including genomic sequencing of all exons, cDNA sequencing and MLPA analysis of these genes in 135 Japanese patients with MFS phenotypes. We also re-evaluated the clinical diagnostic criteria (exclusive of genetic criteria) by a thorough examination of phenotypes including the evaluation of ectopia lentis by ophthalmologists and dural ectasia by radiologists. Molecular study of 104 probands revealed 72 mutations in *FBN1* gene, 4 in *TGFBR1* and 6 in *TGFBR2*. Of the 50 patients who fulfilled the Ghent criteria, 41 (82%) had mutations in *FBN1*, 1 (2%) in *TGFBR1* and 3 (6%) in *TGFBR2*. *FBN1* mutations were also identified in 45 of 68 patients who did not fulfill the criteria, as well as *TGFBR1* in 3 and *TGFBR2* in 5 patients. Major aortic involvement was observed in 76% of patients with *FBN1* mutations, ectopia lentis in 40%, and dural ectasia in 43%. Although most patients showed distinctive skeletal phenotypes, only 14% met the 4 major criteria for skeletal involvement in our study. All patients with ectopia lentis were identified to have *FBN1* mutations, meaning that it has a high diagnostic value. Genotype-phenotype correlation study revealed no significant difference among mutation types in the incidence of aortic involvement, skeletal involvement or dural ectasia, but ectopia lentis was significantly more prevalent in patients with alterations of cysteine residues than in those with other mutation types (p=0.009). In total, out of 100 patients harboring *FBN1* mutations, 41 fulfilled the criteria and 45 did not. 33% (4/12) of patients with *TGFBR1*/*TGFBR2* mutations also fulfilled the Ghent criteria.

THAOS - Transthyretin Amyloidoses Outcomes Survey, A New Global, Web-Based Registry. *R. H. Falk[1], J. Packman[2], D. Grogan[2] for the THAOS Scientific Board* 1) Harvard Vanguard Medical Associates, Boston, MA; 2) FoldRx Pharmaceuticals.

Background: TTR amyloidosis (ATTR) is caused by dissociation of the TTR tetramer into monomers that misfold due to genetic mutations or aging, and ultimately form amyloid deposits in various organs. The >80 known TTR mutations associated with the autosomal dominant disease result in variable phenotypic expressions commonly affecting the peripheral nerves and heart. Symptom onset is in the third to fifth decade of life, consisting primarily of peripheral and autonomic neuropathy, or sixth decade on, presenting with a restrictive cardiomyopathy. Survival for most mutations is 9-11 years after onset, with median survival of 5-7 years in cardiomyopathy patients. Methods: THAOS is a new global, longitudinal observational survey designed to characterize the natural disease history of ATTR, including regional differences in disease expression and the genotypic-phenotypic relationship. Symptomatic and asymptomatic patients with TTR disease will be enrolled and followed for up to 10 years. It is anticipated that several thousand patients will be enrolled across 50 or more survey sites. Patients will continue to receive the current standard of care for their disease, with frequency and type of clinical and laboratory assessments performed according to the physicians practice. Data on cardiac and neurologic examinations, renal/bladder function, quality of life, hospitalizations, medication use, and transplant history will be recorded in the database. Data analysis and publications will be coordinated by the THAOS Scientific Board, an international group of amyloid experts, and complimentary to data collected in the Familial Amyloid Polyneuropathy World Transplant Registry. The THAOS database, a secure interactive web-based system, is an intuitive tool for physicians to enter and review patient data. Results: Identification and initiation of sites in a number of countries is on-going and patient enrollment has begun. Conclusions: Data will be analyzed and reported at least annually. Any physician who cares for these patients is invited to participate. Initial data are expected in late 2008.

The molecular evolution of an inversion polymorphism region 8p23 during human migration. *L. Deng, W. Chen, C. Zeng* Beijing Institute of Genomics, Beijing, China.

Chromosome 8p23.1 is a region of inversion polymorphism found in various populations. Based on HapMap data, previously we identified four haplotype clades in this ~4.7 Mb region, among which clades 1/2 and clades 3/4 correspond to the different orientation respectively [1]. By haplotype analysis we have also speculated that the geographically restricted selection might play a role in the evolution history of this inversion polymorphism. In this study, with the criteria of population size over 20, we analyzed 742 samples of Human Genome Diversity Project (HGDP) from 26 populations in 6 geographic groups, including African (AF), Middle East (ME), European (EU), Southern Asian (SA), Eastern Asian (EA), and Native American (NA). By principle component analysis on genotypes of 1447 SNPs in 8p23, three major haplotype subgroups, named as Hap 1, 2, and 3, were resolved. Previously identified clade 1 and 4 containing HapMap YRI samples exclusively appear to cluster within Hap 1 which mainly consists of African haplotypes also. Hap 2 and Hap 3 correspond to clades 2 and 3 respectively. In addition to these three subgroups among populations, all these 6 geographic groups contain more or less admixture haplotypes in between and the most isolate clusters come from NA and ES samples. As measured by Wrights FST, a clear population differentiation between geographic groups was observed. In particular, NA and EA populations showed considerable genetic difference, suggesting further positive selection events after the migration out of African. Furthermore, multi tests by site frequency spectrum and extended haplotype homozygosity indeed demonstrated strong signatures of positive selection at XKR6 in 8p23 region in these populations. On the other hand, in contrast with AF haplotypes, large diversity was shown in populations from geographic groups of ME, SA, and EU. Although both gene flow and genetic drift could be an explanation for such a phenomenon, most likely the role of complex selection is the mechanism in the maintenance of such unique genetic patterns at 8p23 region. Reference [1] Deng et al. Hum Mutat., 2008 May 12. [Epub ahead of print].

X-linked Cohesin Structural Component SMC1A: Expression and Mechanism of Pathogenicity in Cornelia de Lange Syndrome. *J. Liu[1], R. Feldman[1], Z. Zhang[2], M. Kaur[1], M. A. Deardorff[1,3], D. Clark[1], I. D. Krantz[1,3]* 1) Division of Human Genetics, Children's Hosp Philadelphia; 2) Bioinformatics Core Facility, The Childrens Hospital of Philadelphia; 3) 3 The University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

Cornelia de Lange Syndrome (CdLS) is a dominant genetic disorder with multi-system defects. 60% of probands with CdLS have heterozygous mutations on the *Nipped-B- like* (*NIPBL*) gene, while 5% are due to mutations in the *SMC1A* gene, and 1 patient was found to have mutation in the *SMC3* gene. Cohesin complex is a ring like structure consisting of a SMC1A and SMC3 heterodimer and two non-SMC subunits, it controls sister chromatid cohesion with NIPBL facilitating cohesin loading and unloading. Cohesin complex mediates long distance regulation of gene expression in *Drosophila*, CdLS phenotype is believed to be caused by gene dysregulation rather than chromosomal segregation effects. *SMC1A* is located on the human X chromosome and is reported to escape X inactivation in mice. Our laboratory has identified 22 CdLS probands with *SMC1A* mutations including one male adult. Eleven individuals carry missense or in frame small deletion mutations scattered across the *SMC1A* coding region. Seven mutations are located at a possible mutation hotspot c.1487G>A.Using lymphoblastoid cell lines (LCLs), RT-PCR has revealed both males and females express SMC1A, with females quantitatively expressing twice the amount of *SMC1A* mRNA. There is no difference between patients and controls within the same gender. Allele specific quantitative RT-PCR demonstrated identical levels of expression between the wild type and mutant alleles. Genome wide expression studies did not identify dysregulated *NIPBL* expression among CdLS patients with *SMC1A* mutations, and *vise versa*.Western blotting has showed an equal amount of SMC1A protein expressed among probands and controls. Our study confirms biallelic expression of *SMC1A* in females, and further suggests that mechanistically CdLS is not due to altered levels of *SMC1A* RNA or protein, but rather the produced mutant proteins maintain a residual but reduced function in males and a dominant negative effect in females.

Silver-Russell syndrome with hypomethylation of H19 gene in consanguineous background. *C. Sibille, X. Pepermans, K. Dahan* Center of Human Genetics, Cliniques Universitaires St-Luc, Bruxelles, Belgium.

Silver-Russell syndrome (SRS) is characterized by a pattern of intrauterine growth retardation, postnatal growth with relatively normal head circumference, classical facial phenotype and hemihypotrophy. The most frequent genetic abnormality seen is a uniparental disomy of chromosome 7, found in 7-10% of patients. Recently, a new site of hypermethylation was described, underlying the growing importance of epigenetic factors in clinical genetics. We present the case of a 3 year-old boy born at 39 weeks from consanguineous Moroccan parents with a birth weight of 1,74 kg (-4.7 SD), a length of 42cm (-4.5 SD) and a cranial perimeter of 34.5cm (-1 SD). He was followed for feeding difficulties and bronchial hyperractivity. The clinical examination revealed a dysmorphic child with a prominent forehead, relative macrocephaly, pectus carinatum, lower limbs asymmetry, micrognathism. A verrucous hamartoma was found in the left axillary region. Suspicion of Russell-Silver syndrome was evoked but the search for mUPD7 was negative. A molecular analysis using the ME030BWS/RSS kit by MRC-Holland (the MS-MLPA method) determines of a panel of genes in particular region 11p15. In the present case report, we observe the absence of partial methylation of the H19 gene. Eggermann et al. 2006 identified a loss of methylation in the imprinting domain of *H19* promoter (*ICR1*) in a population of 51 SRS patients, with a frequency 31%. Interestingly, an opposite alteration is found in 10% of cases of Beckwith-Wiedemann syndrome. With hypermethylation of the same maternal region, leading to overexpression of *IGF2*. It seems also that *ICR1* hypomethylated patients have a more typical SRS phenotype than maternal duplicated cases. Exact phenotypical implications stay to be clarified, but body asymmetry and growth retardation are the main clinical features included in our case. Additional Silver-Russell cases in young children are suspected in relatives. Therefore, a genetic counseling and specific familial follow up are ongoing in this family. Search for potential parental unidisomy of *ICR1* is under study.

Genome-wide Association Study of Attention Deficit Hyperactivity Disorder-Combined Type (ADHD-CT); Adjustment for Genetic Heterogeneity in a Large Multicentre Study. *R. J. Anney, M. J. Hill, C. T. O'Dushlaine, E. Kenny, M. Gill, IMAGE consortium* Neuropsychiatric Genetics Research Group, Trinity College Dublin, Institute of Molecular Medicine, Trinity Centre for Health Sciences, St. James' Hospital, James' Street, Dublin 8 Ireland.

As modern humans have spread throughout the world, allele frequencies and linkage disequilibrium (LD) have become more varied between populations. Population admixture is a major source of bias in the case-control study design. Family-based designs, such as the Transmission Disequilibrium Test, can be used to limit this bias. However, the power of the TDT to detect disease susceptibility loci (DSL) can also be influenced by population admixture through its impact on the degree of LD between the genetic marker and the DSL. We have examined the population clustering of a large multicentre study of approximately 950 ADHD-CT families collected from the European population. We have applied selection criteria to try to reduce heterogeneity at the clinical and genetic level. We present data from this pre-cleaning step and discuss the implications to large multicentre studies. Moreover, we present the results of the application of this data to GWAS data using data generated from the IMAGE ADHD-CT study.These data are examined at the marker, gene and hypothesis-free and hypothesis-driven gene-network analysis. Moreover, we examine the functional variation of genes tagged by associated SNP markers.

Idiopathic Nephrocalcinosis: Possible Genetic Causes. *G. Nesterova, W. J. Introne, G. A. Golas, C. Ciccone, M. Huizing, W. A. Gahl* Medical Genetics Branch, NHGRI, NIH, Bethesda, MD.

Nephrocalcinosis (NC) consists of calcium deposition mainly within the medullary portion of the kidneys. Many known etiologies of secondary NC exist, including Dents disease, exogenous and endogenous forms of hypervitaminosis D, distal renal acidosis, hyperparathyroidism and others. In addition, many patients with renal calcifications have isolated, idiopathic NC, sometimes causing significant renal impairment. Some of these patients could have disorders of vitamin D metabolism not yet discovered. We evaluated two unrelated patients (ages 5 years and 12 years) at the National Institutes of Health with hypercalciuria and isolated nephrocalcinosis. Both children have high normal serum calcium, mildly elevated serum vitamin D_3 levels, and undetectable parathyroid hormone. Neither child has evidence of rickets or a history of excessive vitamin D_3 consumption. Given the high vitamin D levels despite high normal serum calcium, we hypothesize that the underlying mechanism involves dysregulation of vitamin D_3 metabolism. The enzyme 1-alpha-hydroxylase (CYP27B1) converts the inactive form, 25-dihydroxyvitamin D_3 to the active form,1-,25 dihydroxyvitamin D_3(1- 25$(OH)_2D_3$). This biologically active form plays a critical role in intestinal and renal calcium absorption, as well as in binding to the vitamin D receptor (VDR) within distal tubules of the kidney, further regulating CYP27B1 gene expression. 1-,25$(OH)_2D_3$ is inactivated by the enzyme 24-hydroxylase (CYP24). A loss of function mutation within this gene could theoretically lead to increased 1-,25$(OH)_2D_3$. Two additional molecules critical for calcium transport along the cells of the distal tubule are VDR and the active calcium transporter, TRPV5. Our initial investigation has targeted the enzymes responsible for regulating the active vitamin D_3, CYP24 and CYP27B1. Sequencing of both these genes is currently underway. Sequence analysis of the VDR and TRPV5 genes could be the next step.

Reporting cystic fibrosis (CF) modifier gene effects to study participants: Pilot survey. *R. Hayeems[1], F. Miller[1], R. Christensen[1], N. Anderson[2], M. Corey[2], J. Zielenski[2], P. Durie[2], The Canadian Consortium for CF Genetics Studies* 1) Dept Health Policy Management Evaluation, University of Toronto 155 College Street, 4th Floor, Toronto, ON M5T 3M6; 2) The Hospital for Sick Children 555 University Avenue, Toronto, ON M5G 1X8.

Background: Some argue that the principle of respect for persons obligates researchers to proactively disclose genetic research results to participants. Others contend that these results are inherently provisional and that in the context of a research relationship, this is an inappropriate obligation. Unlike the scholarly commentaries fueling this debate, this novel pilot study used an empiric approach. **Methods:** Following academic publication reporting the co-modifying effect of MBL-TGFB1 genes on lung function among a pediatric CF population (Dorfman et al 2008), a lay newsletter and survey were sent to a sample of this study population to explore their understanding of this discovery as well as their expectations of research related disclosure. **Results:** While 84.3% of 115 respondents reported that the newsletter was easy to understand, 60% had remaining questions about the discovery. Only a slim majority correctly agreed that this discovery was related to the *average* severity of CF disease for the participants in this study and correctly disagreed that this discovery was related to the severity of CF disease in an *individual* (53.9%, 54.8%, respectively). The majority of respondents (74.8%) desired more detailed information and 87.0% felt researchers are always obligated to communicate discoveries to participants. While not statistically significant, trends indicate that participant expectations may be driven by misunderstanding the nature of the discovery, a belief that researchers are indeed obligated to report discoveries, or by individual characteristics (e.g. being a parent, being female, having more severe CF disease). **Discussion:** While participant misunderstanding and their perception of researchers obligations to report results may be driving expectations, whether these factors actually justify such an obligation remains unanswered by these data and warrants further empiric consideration.

Phase 2 clinical trials of the pharmacological chaperone AT1001 for the treatment of Fabry disease. *R. Schiffmann[1], D. P. Germain[2], J. Castelli[3], A. Shenker[3], D. J. Lockhart[3]* 1) Institute of Metabolic Disease, Baylor Research Institute, Dallas, TX, on Behalf of the AT1001 Study Group; 2) Université de Versailles, Hôpital Raymond Poincaré, Garches, France; 3) Amicus Therapeutics, Cranbury, NJ.

PURPOSE: AT1001 (migalastat hydrochloride) is an orally administered, small molecule pharmacological chaperone designed to selectively bind -galactosidase A (-Gal A), thereby increasing the enzymes stability, trafficking to the lysosome, and cellular activity. METHODS: 18 men and 9 women between the ages of 17 and 65 were enrolled in four open-label, multinational Phase 2 trials designed to evaluate the safety, tolerability and effects of AT1001 given at different doses and dose regimens. Twenty-six subjects completed a primary treatment arm and all entered the optional treatment extension. Seventeen subjects have been treated with AT1001 for at least 48 weeks and 6 subjects have been treated for more than two years. RESULTS: AT1001 was generally safe and well-tolerated at all doses evaluated. Twenty-four of the 26 subjects demonstrated an increase in -Gal A as measured in leukocytes, kidney, and skin. The average leukocyte -Gal A increase observed in males was greater than four fold. Kidney globotriaosylceramide (GL-3) levels as measured in urine or biopsies were decreased in subjects who demonstrated greater increases in levels of -Gal A. Renal and cardiac function results were encouraging, including data in subjects after 96 weeks of treatment. Subjects in vivo -Gal A responses were consistent with predictions based on in vitro testing of Fabry mutations, suggesting a pharmacogenetic approach may be used to select likely responders for future studies. CONCLUSION: These data suggest AT1001 merits further investigation as a potential therapy for Fabry disease.

Gene variants associated with respiratory distress syndrome in newborns. *H. Heins, J. Wambach, D. Wegner, P. An, A. Muzzarelli, P. Yang, L. Walther, F. Cole, A. Hamvas* Washington University, St. Louis, MO.

Respiratory distress syndrome (RDS) in newborns is a complex disease. To identify genes associated with RDS, we genotyped 588 tag SNPs for 38 candidate genes in 7 pathways of lung function and 83 ancestry informative SNPs in unrelated European American (EA, n=344) and African American (AA, n=286) infants with and without RDS. We used STRUCTURE to estimate genetic admixture. We tested SNP association with the risk of RDS using logistic regression adjusted for gestational age, gender, and admixture. P-values were corrected for multiple tests using a revised false discovery rate approach. Among EA infants, a variant in an epithelial sodium channel (SCNN1G) was independently associated with RDS (P=0.01). Among AA infants, 5 variants in 5 genes along different pathways were independently associated with risk for RDS (P-SNP, Table). Effect size for each variant was moderate (r^2). Gestational age and sex were consistently significant predictors; genetic admixture was not. We conclude that variants in genes expressed in the lung confer risk for newborn RDS. We speculate that epistatic interactions among these genes contribute to diverse mechanisms and outcomes of RDS.

Gene	Function	rs #	Location	# tags	Race	r^2 (%)	P-SNP
ACE2	ACEreceptor	1514280	Intron 14	0	AA	10.8	.009
SCNN1B	sodium flux	1004749	Intron 1	0	AA	11.4	.007
NAPSA	protease	1274517	Intron 1	1 (I40T)	AA	10.1	.013
ABCA3	transport	150926	Intron 28	3	AA	10.6	.009
FGF7	structure	4480740	Intron 11	5	AA	12.0	.002

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Strong evidence of epistatic interactions involving NOS1AP in schizophrenia. *L. M. Brzustowicz[1], Y. Huang[2], V. Saviouk[1], A. S. Bassett[3], V. J. Vieland[2]* 1) Dept Genetics, Rutgers University, Piscataway, NJ; 2) Battelle Center for Mathematical Medicine, The Research Institute at Nationwide Childrens Hospital, Columbus, OH; 3) Dept of Psychiatry, University of Toronto, ON.

We have previously shown linkage between 1q23 and schizophrenia and linkage disequilibrium (LD) with markers within NOS1AP in a set of Canadian families of European descent. We have reported significantly increased expression in schizophrenia of NOS1AP in postmortem brain samples. We have shown that rs12742393 is in strong LD with schizophrenia, and that the associated allele can significantly increase expression from the NOS1AP promoter in a cell culture system. To search for possible epistatic interactions with NOS1AP we have reanalyzed our microsatellite genome scan data from these families conditioning on rs12742393 genotype. A baseline scan without conditioning produced four peaks (1p, 2p, 13q, 17q) with Posterior Probabilities of Linkage >20% in addition to the peak at NOS1AP (PPL=97%). Excluding the region around NOS1AP, the conditional analysis produced 8 peaks with a PPL>20%. In addition to the baseline scan peaks, new peaks were seen on 2q, 7p, 9q, and 12q. The location of the 12q peak is near NOS1, a known binding partner of NOS1AP. The baseline scan produced a maximum PPL of 6% in this area, increasing to 36% in the conditional scan, indicating epistasis. We have begun evaluating this interaction in other datasets. Samples of Chinese ancestry from the NIMH HGI collection were genotyped for rs12742393, but showed no evidence of LD (PPLD<2%). Analysis of linkage data from 12q in this sample produced a baseline PPL of 4%, rising to 10% in the conditional analysis. Sequential updating of the conditional results for the Canadian and Chinese datasets produced a final PPL of 63%, indicating that both datasets support the genetic interaction of NOS1AP with a 12q locus. Our results demonstrate that epistatic interactions can obscure linkage and association signals, leading to inconsistent replication across data sets. Despite this, we have demonstrated strong evidence of gene x gene interaction between NOS1AP and additional schizophrenia susceptibility loci.

DNA methylation surveying of the Major Histocompatibility Complex - a twin study. *K. Gervin[1], H. E. Akselsen[1], M. Hamerø[1], R. Moe[1], J. Harris[2], D. E. Undlien[1,3], R. Lyle[1]* 1) Department of Medical Genetics, Ullevål University Hospital, Oslo, Norway, Oslo, Norway; 2) Division of Epidemiology, Norwegian Institute of Public Health, Oslo, Norway; 3) Institute of medical genetics, Faculty Division Ullevål University Hospital, University of Oslo, Oslo, Norway.

Phenotypic differences between individuals are an outcome of genetic (DNA sequence) and epigenetic (DNA methylation/histone modifications) variation. Whereas variation at the genetic level has been studied extensively, little is known about epigenetic variation. Disturbance of DNA methylation leading to aberrant gene expression has been shown to be involved in many diseases, and variation in DNA methylation may be a contributing factor in common diseases. However, much is still unknown regarding the mechanism, variation and biological function of DNA methylation. In this study we use monozygotic (MZ) and dizygotic (DZ) twins to explore variation, heritability and patterns of DNA methylation in the classical major histocompatibility complex (MHC). The MHC is a gene-dense and highly polymorphic region on human chromosome 6p21.3, containing genes with a broad range of functions within the innate and adaptive immune systems. We performed bisulphite sequencing of 1670 CpG sites in 190 regions in the MHC in 50 MZ and 50 DZ healthy Norwegian twin pairs. We isolated lymphocyte subpopulations (CD19+, CD8+, CD4+ and CD4+CD25+) and study single-cell types to overcome the problem of epigenetic heterogeneity in whole blood. Regions of interest include CpG islands, other CG-rich sequences, the 5end of genes not associated with defined CpG islands and conserved non-coding regions. Individuals show significant variation in DNA methylation both between and within regions. In addition, many regions have a complex pattern of variation. Globally, there appears to be a bimodal distribution of DNA methylation, with regions tending to show either low or high methylation. Interestingly, intra-pair differences are generally lower than inter-pair differences, and MZ pairs show lower intra-pair differences than DZ pairs, indicating a level of genetic control of DNA methylation variation.

The Genetics Of Prostate Cancer - Is It Time To Consider A New Cancer Hypothesis? *B. Gottlieb[1,2], L. K. Beitel[1,2,3], M. Trifiro[1,3]* 1) Dept Cell Genetics, Lady Davis Institute Medical Research, Montreal, PQ, Canada; 2) Dept. Human Genetics, McGill University, Montreal, PQ, Canada; 3) Dept. Medicine, McGill University, Montreal, PQ, Canada.

We investigated the genetics of prostate cancer by examining the androgen receptor gene (*AR*) in both diseased and non-diseased prostate tissues and comparing them to *AR*s in other androgen-responsive tissues and blood. In both diseased and non-diseased prostatic tissue, we identified as many as 10 variants, of a well-known functional polymorphism (a CAG repeat) of the *AR*. In almost all cases the CAG repeats were shorter and therefore the AR proteins were more active than normal. In addition, the majority of these variants were present in very small quantities, which we have termed minority variants. In contrast, in blood and other androgen-sensitive tissues only a single variant was found. The presence of multiple *AR* variants in non-diseased prostate tissues, further suggests that *AR* could play a role in the increased susceptibility of prostate tissue to cancer. Based on these observations we are proposing a new cancer hypothesis in which the critical carcinogenic-promoting event is selection as opposed to current mutation-based hypotheses. In our theory one or more minority *AR* variants may replace the majority normal variants in prostate tissues due to strong selection pressures, as a result of altered environmental conditions or epigenetic factors. This hypothesis could then explain why environmental factors such as diet and exercise are found to be risk factors for prostate cancer, even though they cannot be considered mutagenic agents. Of more immediate interest is its relevance to treatment, as this hypothesis could explain how it is that almost all prostate tumors, while initially responding to anti-androgen-based chemotherapy, become resistant to therapy due to becoming androgen-independent, often within a relatively short time period. According to our theory anti-androgen therapy is likely to result in the selection of the shorter *AR* CAG repeats in tumors, which would be functionally much more responsive to low androgen levels, and so still be able to grow.

Large non-recurrent CNVs in children with autism spectrum disorders from a founder population. *K. Rehnström[1,2], E. Jakkula[1,3,4], H. Kilpinen[1], M. Rossi[1], O. P. H. Pietiläinen[1], L. von Wendt[5], L. Peltonen[1,2,4,6]* 1) National Public Health Institute and Institute for Molecular Medicine Finland (FIMM), Helsinki, Finland; 2) Univ of Helsinki, Finland; 3) FIMM, Finnish Genome Center, Univ of Helsinki, Finland; 4) Broad Institute of Harvard and MIT, Cambridge, MA, USA; 5) Unit of Child Neurology, Hospital for Children and Adolescents, Helsinki, Finland; 6) Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK.

Autism spectrum disorders (ASDs) are a group of severe, childhood-onset neuropsychiatric disorders. Twin- and family studies have shown a strong genetic component in ASDs but few, if any, common predisposing variants have been identified and convincingly replicated. Recent reports of have implicated copy number variants (CNVs) as significant predisposing factors in ASDs. In an effort to address potential enrichment of rare alleles predisposing to ASDs among Finns, we have genotyped 164 patients with ASDs from Finnish families using Illumina HumanHap 300 and HumanHap 550 BeadChips. A subset of 54 patients originate from an internal subisolate in Central Finland, and several families have been genealogically linked further reducing the genetic heterogeneity. The GWA data did not show significant enrichment of shared homozygosity or haplotype regions among patients. We next used the dataset to monitor for potential enrichment of sizeable chromosomal aberrations We used QuantiSNP to call large CNVs, and identified several large chromosomal aberrations but again no enrichment of large CNVs across families. Among 164 cases we identified 8 CNVs 1-2.5Mb in size. These included 4 individuals with amplifications of 15q11-12, a chromosomal region well known as a predisposing locus for ASDs. Other large aberrations included duplications spanning 1Mb or more on 1q42, 9q21, 22q11 and a deletion spanning approximately 1Mb on 22q13. The relevance of these findings for ASD was further confirmed in the GWA dataset of a population cohort of ~5000 Finns. Based on this data we conclude that even in a founder population, with genealogically linked individuals, the causes for autism are heterogenous, and only evidence for non-recurrent large CNVs could be established.

Is the thrifty genotype hypothesis (TGH) supported by evidence based on confirmed type 2 diabetes (T2D) susceptibility variants? *L. Southam[1], N. Soranzo[2], SB. Montgomery[2], K. Chapman[1], MI. McCarthy[1], I. Barroso[2], E. Zeggini[1]* 1) WTCHG, University of Oxford, Oxford, UK; 2) WTSI, Hinxton, UK.

The TGH suggests that the high prevalence of T2D may be partly due to common genetic variants that have risen in population frequency due to natural selection having favoured alleles that were once advantageous in periods of famine. The recent detection of 18 robustly replicating T2D susceptibility loci enables us for the first time to look for evidence in support (or otherwise) of the TGH using proven loci. We have examined several characteristics that might indicate evidence for selection at the 18 loci: we have determined whether the risk allele at index single nucleotide polymorphisms (SNPs) is the derived or ancestral allele and whether the risk allele is the major or minor allele (in terms of allele frequency) in the reported studies. We find that the risk allele is the ancestral allele at 12 loci, compared to 9 expected by chance (binomial test p=0.12). We also find no significant difference between risk and non-risk allele frequency; 10 risk alleles are major and 8 minor (p=0.41). We determined the integrated haplotype score (iHS) statistic for each locus. Two loci had iHS > 2: rs10923931 (NOTCH2) & rs8050136 (FTO). One locus had an iHS < -2: rs7901695 (TCF7L2). The extended haplotype is associated with the risk allele at rs8050136 (FTO) only. Finally, we assessed the population differentiation statistics FST for the 18 loci for all pairwise comparisons of 11 worldwide populations genotyped by the HapMap phase 3 and compared them to the neutral expectation, defined as the 95% quantile of the empirical FST distribution calculated over 1.5 Million genome-wide loci. We found an excess of high FST values for rs5215 (KCNJ11) in African populations and rs7901695 (TCF7L2) in East-Asian populations (both p=0.019), indicating possible diversifying selection at these loci. Our results do not provide conclusive evidence to support the TGH. However, the number of robustly replicating T2D loci identified to date is small. In addition, the causal variants for most susceptibility loci have not yet been determined -this could affect the SNP-specific analyses we have carried out.

Analysis of the CNTNAP2 Gene in Autism. *J. L. Haines[1], N. Schnetz-Boutaud[1], K. Brown[1], K. Gainer-Luci[1], M. L. Cuccaro[2], J. R. Gilbert[2], M. A. Pericak-Vance[2]* 1) Center for Human Genetics Research, Vanderbilt Univ Medical Center, Nashville, TN; 2) Miami Institute for Human Genomics, Univ. Miami, Miami, FL.

Autism Spectrum Disorders (ASDs) encompass several diagnoses with the three most prevalent being autism, Aspergers Syndrome and Pervasive Developmental Disorder (PDD-NOS). ASDs are complex neurodevelopmental disorders with an onset early in childhood and ~4:1 male:female ratio. The ASDs are characterized by impairments in language and reciprocal social interactions, combined with repetitive and stereotypic behaviors. Concordance rates for MZ twins have been estimated between 60%-90% and 0%-10% for DZ twins, suggesting a strong genetic etiology. Despite strong evidence of a genetic component and substantial efforts, very little of the genetic etiology has yet been explained. Based on the results of a Genome Wide Analysis Study (GWAS) of autism, the Contactin Associated Protein Like-2 (CNTNAP2) gene on chromosome 7q was implicated in autism risk, and has already been confirmed in 2 additional datasets. We examined two previously associated CNTNAP2 SNPs in our independent dataset of 403 Caucasian multiplex and trio families whose affected individuals meet DSM-IV and ADI criteria for autism. Analysis of the overall dataset generated a p-value of 0.03 using the pedigree disequilibrium test (PDT) for RS7794745, confirming the previous results. To test for the specificity of the results we examined two subsets of the data. This result was stronger in the subset of male-only families (p=0.004, N=303), and in the severe subset of families where the probands has >50%tile scores on each of the subdomains of the ADI (p=0.003, N=96). There was no enrichment of the male-only families in the severe subset. These results confirm (1) that variations in CNTNAP2 are broadly important in ASDs; and (2) that GWAS is a powerful tool to unravel the genetics of even behaviorally complex traits. The specificity of these results lends further credence to the concept that multiple genes likely contribute to different subphenotypic expressions of ASDs.

Multidisciplinary Clinic for BRCA1/2 Carriers at Guys Hospital, London: Experience after 24 months. *L. P. Izatt[1], C. Jacobs[1], C. Firth[1], C. Langman[1], H. Hamed[1], A. Tutt[1], S. Rose[1], S. Hyams[1], G. Pichert[1], I. Jacobs[2], U. Menon[2], Guys & St. Thomas Charity* 1) Clinical Genetics, Guy's and St.Thomas' NHS Foundation Trust, London, UK; 2) Women's Institute, University College, London, UK.

BRCA1/BRCA2 carriers have increased breast & ovarian cancer risks & face complex decisions over ca risk management strategies, including risk-reducing (RR) surgery, & surveillance. Our multidisciplinary clinic (MDC) for BRCA1/2 carriers is a new service that has run for 24 months. All patients would see a geneticist & could choose to see an oncoplastic br surgeon, oncologist, gynae-oncologist, or psychologist in a one-stop clinic. All patients were discussed at a MDT meeting before the clinic to co-ordinate best practice care in conjunction with local clinicians. **Aims** To offer all BRCA1/2 carriers an annual MDC review, to address their needs, update them on new developments, & invite participation in research trials. We audited uptake & satisfaction of the MDC, the impact of the service on uptake of surveillance, RR surgery & research trial entry. **Results** 79% (201/254) patients invited were interested in attending the MDC. Of 170 BRCA1/BRCA2 carriers seen, 8 were male. The age range was <20 to >70, median 40-50. Patients were seen by:-Geneticist/genetic counsellor, 170, br. surgeon, 95, gynae-oncologist, 103, oncologist, 13, clinical psychologist, 64, research nurse, 130. Recommended br surveillance was altered in 44% (65/147) eligible women after MDC with 2 screen detected brca during f/u. 27% (42/157) patients had RR breast surgery prior to MDC and 54% (62/115) were considering/had decided to have RR mastectomy after. 33% (45/134) patients had bilat. oophorectomy before the MDC & 85% (76/89) patients were considering/ had decided to have bilateral salpingo-oophorectomy after. Recruitment to the EMBRACE trial rose from 17% (29/170) before MDC to 84% (114/141) after. Questionnaires completed by 76% of attendees showed high satisfaction, with a median 9 out of a 1-10 scale, citing the one-stop facility as key. **Conclusion** Our MDC is a novel, successful one-stop clinic leading to improved patient-centred care with increased uptake of cancer risk reducing strategies & research trial participation.

A silent variant in the *WAS* gene causing Wiskott-Aldrich syndrome. *M. J. van Belzen[1], M. W. Boogaard[1], M. M. ten Dam[2], M. Losekoot[1], R. G. M. Bredius[2]* 1) Clinical Genetics, Laboratory for Diagnostic Genome Analysis, Leiden University Medical Center, Leiden, The Netherlands; 2) Pediatrics, Division of Immunology, Hematology, Oncology, Bone Marrow Transplantation and Autoimmune Diseases, Leiden University Medical Center, Leiden, The Netherlands.

Wiskott Aldrich syndrome (WAS) is a rare X-linked disease, characterized by microthrombocytopenia, eczema and immunodeficiency causing recurrent infections, a high incidence of autoimmunity and of lymphoid malignancies. The disorder is caused by mutations in the *WAS* gene, in which over 300 mutations have been described. Mutations include nonsense, frameshift and missense mutations that occur over the entire coding region. In addition, several splice site mutations have been described, mainly occurring at the exon/intron boundaries. The effects of *WAS* mutations on the function of the WAS protein (WASp) are variable, resulting in a wide range of phenotypes. The case presented here is a one year old boy with a clinically moderate phenotype of the Wiskott-Aldrich syndrome (Zhu score: 3 to 4, pre-stem cell transplantation (SCT)). Sequence analysis of the entire coding region of the *WAS* gene revealed only one silent variant in exon 7: c.687G>T, p.Gly229Gly. Analysis with splice predict software indicated the creation of a strong donor splice site in the middle of exon 7. RNA studies were performed to determine whether this new splice site was active. The major RNA transcript in blood of the patient lacked the last 49 bp of exon 7, resulting in a frameshift and the introduction of a premature stop codon: r.686_734del, p.Lys230MetfsX15. Flowcytometry and western blot analysis of WASp showed that this protein could not be detected in the patients blood, thereby confirming the pathogenicity of the silent DNA variant. In conclusion, we have shown that a silent variant in the *WAS* gene can cause classical Wiskott-Aldrich syndrome, a mechanism which has not been reported before in Wiskott-Aldrich patients.

3.8 MB deletion of chromosome 5q in a newborn tracheal agenesis patient. *E.de Jong[1,2], H. Douben[1], B. Eussen[1], D. Tibboel[2], A. de Klein[1]* 1) Department of Clinical Genetics, Erasmus Medical Center, Rotterdam, Netherlands; 2) Department of Pediatric Surgery, Erasmus Medical Center - Sophia Children's Hospital, Rotterdam, Netherlands.

The department of Pediatric Surgery, in collaboration with the department of Clinical Genetics has collected a large cohort of patients with tracheal and foregut-related anomalies. The most prevalent are Esophageal Atresia (EA) with or without Tracheo-Esophageal Fistula (TEF), associated with VACTERL anomalies (Vertebral, Anal, Cardiovascular, Tracheo-Esophageal, Renal and Limb) occurring in around 25% of patients. Tracheal agenesis (TA), a congenital anomaly of the respiratory tract with an incidence of 1 in 50,000 births, is less frequent. Routinely, EA/TEF and also TA patients are karyotyped and screened for subtelomeric aberrations with MLPA (Multiplex Ligation-dependent Probe Amplification). Array-CGH (105K Agilent) was used to screen for small deletions or duplications in cell lines (n=6) and paraffin embedded tissues (n=6) of twelve TA patients. Our TA patients showed a variety of copy number variations of which many were parental inherited variants. In one of our TA patients we observed a 3.8 MB deletion on chromosome 5q11.2, not present in the mother: the father was not available. This 3.8 MB deletion was confirmed with FISH and Q-PCR. The deleted region contains 17 genes and we are currently investigating, using prioritizing programs, whether we can relate any of these genes to the TA or other phenotypes, since in addition to TA this patient also suffered from other foregut-related anomalies, such as cartilage rings in the esophagus, aberrant right bronchus and rib abnormalities.

Molecular characterization of a large cohort of Cornelia de Lange Syndrome Italian patients and related phenotypes. *A. Selicorni[1], S. Russo[2], C. Gervasini[3], M. Masciadri[2], P. Castronovo[3], A. Passarini[1], D. Milani[1], L. Larizza[2,3]* 1) Pediatric Dep.Fondazione Ospedale Maggiore Policlinico Mangiagalli Regina Elena Milan Italy; 2) Molecular Genetics Istituto Auxologico Italiano Milan Italy; 3) Medical Genetics San Paolo School of Medicine University of Milan Italy.

Cornelia de Lange syndrome (CdLS) is a rare multiple congenital disease characterized by facial dysmorphisms, growth/mental retardation, hirsutism, small hands and feet or upper limb reduction defects. Mutations in the NIPBL gene (5p13.2) are responsible of about half of CdLs cases while a small percentage of pts shows molecular defects in the SMC1L1 gene (Xp11.22). SMC3 gene has been associated with CdLS, although its impact on the CdLS syndrome remains to be defined. We report on the thorough molecular characterization of major genes and submicroscopic genomic rearrangements in a clinically heterogeneous sample of 97 Italian CdLS pts. Mutational screening of NIPBL, carried on by DHPLC and direct sequencing identified 7 missense, 1 in frame deletion, 11 splice-site, 9 nonsense and 13 frameshift mutations, while application of MLPA led to disclose three large multiexon deletions. In selected cases transcript analysis showed the limits of mutation classification at the genomic level, as in two prenatal diagnoses in which the mutation observed in a previous child turned out to be a splicing defect (Q1640Q substitution) or was confirmed to lead to missplicing (c.6108+6T->G, causing the skip of exon 34).Two SMC1L1 mutations were detected, among 36 NIPBL-negative pts and one genomic imbalance and two CNV were disclosed by array-CGH among 24 NIPBL-SMC1L1-negative cases. The stepwise application of this molecular diagnostic flow-chart enabled to disclose the molecular lesion in 51/97 patients (52,5%) also providing a selected cohort of pts to be investigated for candidate genes. Increasing numbers of molecularly characterised CdLS pts should permit to address genotype-phenotype correlations. Even in the case of NIPBL inactivating mutations, the degree of mental retardation is highly variable, due to different effects of the mutation depending on early or late truncation, and to other unknown genetic and epigenetic factors.

Ofd1 limb mesenchymal inactivation results in abnormal anteroposterior patterning and shortening of long bones. *S. Bimonte[1,2], L. Quagliata[1], R. Tammaro[1], M.-G. Ascenzi[3], B. Franco[1,4]* 1) Telethon Institute of Genetics and medicine, TIGEM, Naples, Italy; 2) European School of Molecular Medicine (SEMM), Naples; 3) Department of Orthopaedic Surgery, Biomechanics Research Division, University of California, Los Angeles; 4) Department of Pediatrics, Medical Genetics Services, University Federico II, Naples.

Oral-facial-digital type I (OFDI) syndrome is an X-linked dominant male lethal developmental disorder characterized by oral, facial and digital abnormalities. Recent data ascribed OFDI to the growing number of diseases due to dysfunction of primary cilia. Ofd1 null mutants recapitulate the phenotype observed in OFDI patients and displayed skeletal defects. To overcome the embryonic male and perinathal female lethality observed in Ofd1 null mutants we have generated a conditional model with Ofd1 limb mesenchyme specific inactivation. These mice displayed a severe polydactyly with loss of antero-posterior digit patterning, aberrant cilia formation, and shortening of long bones. Defective digit pattering was found to be associated to progressive loss of Shh signaling and impairment of Gli3 processing. Shortening of long bones was found to be associated to defective Ihh signalling and to abnormalities of proliferating chondrocytes as revealed by RNA in situ studies. Finally Von kossa staining and RNA in situ studies demonstrated defective bone mineralization accompanied by loss/reduction of bone collar development suggesting a defect in osteoblast differentiation. Intriguingly, IF analysis demonstrated that cilia are present although shorter than those observed in wild-type animals. This data suggest that, in the limb bud, Ofd1 is not essential for cilia outgrowth although its presence is necessary for formation of normal cilia, contrary to what observed at the embryonic node of Ofd1 null animals. Altogether our data demonstrate that Ofd1 is a pattering factor that plays multiple roles in limb and endochondral bone development.

Genome-wide association study of intraocular pressure in 1304 individuals. *S. M. Hosseini[1], D. Waggott[2], A. P. Boright[3], S. B. Bull[2], W. Sun[4], P. A. Cleary[4], A. D. Paterson[1], DCCT/EDIC Research Group* 1) Genetics and Genome Biology, Hospital for Sick Children, Toronto, Canada; 2) Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Canada; 3) Department of Medicine, University of Toronto, Toronto, Canada; 4) Biostatistics Center, The George Washington University, Rockville, MD.

Background: Intraocular pressure (IOP) is clinically important as one of the primary risk factors for the common form of glaucoma. Diabetes is associated with an increase in both IOP and glaucoma. Twin and family studies show a genetic basis for both glaucoma and IOP. Identifying genetic variants associated with IOP might shed light on pathophysiological mechanisms of glaucoma. **Purpose:** To identify quantitative trait loci associated with IOP in Diabetes Control and Complications Trial (DCCT) cohort **Methods:** DCCT was a clinical trial to compare the effect of intensive and conventional diabetes treatment on the development and progression of complications. IOP has been measured annually in DCCT probands using applanation tonometry. DNA samples from the probands (n=1419) was genotyped using Illumina 1M beadchip. After removing poor performing SNPs and outliers based on population stratification, genotype data was available for ~842K SNPs with a minor allele frequency 1%; on 1304 white probands. Primary non-parametric statistical analysis using a univariate model was performed on IOP measurements as a continuous trait at DCCT baseline. **Results:** Several SNPs show evidence of association with IOP (5 with a P-value 10^{-5}).Of these rs8077655 on 17q (P-vlaue= 9.61E-6) coincides a linkage peak for primary open angle glaucoma (Wiggs et al, 2000). rs177667 (P = 2.31E-6) is located on 7q within a locus recently linked to IOP (Duggal et al, 2007). Collaborative efforts to replicate these primary results are underway. **Conclusion:** This genome-wide association study of intraocular pressure identified a number of interesting loci, some of which present in regions linked to IOP or glaucoma. More comprehensive analysis of IOP data including the use of repeated IOP measurements are underway and may increase the power to detect modest genetic effects.

Concerns of South Korean Patients and Family Members Affected with Genetic Conditions: An Interpretive Analysis. *H. Kang[1], P. McCarthy Veach[2], B. LeRoy[1]* 1) Institute of Human Genetics, University of Minnesota, Minneapolis, MN; 2) Educational Psychology, University of Minnesota, Minneapolis, MN.

Genetic counseling is expanding globally. However, many countries such as South Korea lack healthcare professionals specifically trained as genetic counselors. Currently in South Korea, nurses working in genetics clinics provide some services: also several websites are devoted to genetic conditions. To date, no one has empirically examined needs of South Korean patients and family members who have genetic concerns. Therefore, the present study investigated patients and family members general concerns by accessing and analyzing messages posted to the websites. Eighteen websites were accessed: 1 concerns general genetic conditions and 17 concern specific conditions [e.g., Muscular Dystrophy (3 sites), ataxia, Down syndrome, Rett syndrome, Turner syndrome, Ehlers Danlos, Treacher Collins syndrome, Wilson, MPS, Angelman syndrome, Osteogenesis Imperfecta, dwarfism, RP, VCFS, Charcot Marie Tooth). A random sample of 700 messages posted to the websites message boards was obtained. Messages were translated into English, analyzed and audited using interpretative content analysis. Messages were written by patients and family members; most were detailed and specific. Three major themes and 33 categories were extracted: 1) Medical Questions (e.g., clinical symptoms, diagnosis, prognosis, recurrence risk, prevention, inheritance); 2) Management Needs (e.g., therapy and treatments, education, nutrition, medical facilities and devices, parenting issues, financial support, folk remedies); 3) Psychosocial Issues [e.g., emotions (fear, worry, sadness, disbelief, depression, etc.), physical and psychological depletion, social issues (stigma, secrecy, etc.), and need for social support]. The tone of the messages suggests that writers were sincerely seeking help and would trust the responses that they received. Message content further indicates that most writers lacked basic genetic/medical information, resources are limited, and there are unique cultural issues. Representative messages, unique cultural features, and practice and research recommendations will be presented.

The assessment of confounding in genetic epidemiology may not be reliable in cases of low minor allele frequency. *A. Yesupriya, R. M. Ned, M. H. Chang, N. F. Dowling for the CDC/NCI NHANES III Genomics Working Group* National Office of Public Health Genomics, CDC, Atlanta, GA.

The confounding of associations by environmental risk factors or population structure may affect the validity of study findings in genetic epidemiology. Appropriate methods for identifying confounders include assessing if the odds ratio that is adjusted for potential confounders differs from the unadjusted odds ratio by some pre-specified amount (e.g., 10%). However, adjusted and unadjusted odds ratios may also diverge if these estimates are unstable. Previous studies have reported that estimates of effect may be unstable for genetic variants when the minor allele frequency (MAF) is low (e.g., <10%). We examined if MAFs were correlated with the percent difference (absolute value) between adjusted and unadjusted odds ratios using simulated data and genetic data from the Third National Health and Nutrition Examination Survey (NHANES III). Data for the simulation study included 1000 single nucleotide polymorphisms from a hypothetical study of 1000 cases and 1000 controls. Data from NHANES III was used to examine the association of 33 candidate genetic variants and chronic kidney disease. Odds ratios were calculated from logistic regression models. Adjusted models included two randomly generated dichotomous covariates for the simulation study and age (<50, 50 years) and sex for the NHANES III study. Both additive and co-dominant modes of inheritance were considered. We found that MAFs were correlated with the percent difference between the adjusted and unadjusted odds ratios for the additive ($p< 0.01$) and co-dominant modes of inheritance (heterozygous contrast: $p=0.014$; homozygous contrast: $p< 0.01$) in the simulation study. In the NHANES III study, we found that this relationship was significant for the additive mode of inheritance ($p< 0.01$) and the homozygous contrast of the co-dominant mode of inheritance (p 0.02). We show that MAFs can be associated with the percent difference between adjusted and unadjusted odds ratios. We conclude that the evaluation of confounding may not be reliable in genetic association studies when minor allele frequencies are low.

Meta-analysis of genome-wide association studies identifies many additional height loci and highlights new biological pathways in human growth. *G. Lettre[1,2], M. N. Weedon[3], H. Lango[3], GIANT Consortium* 1) Broad Institute, Cambridge, MA; 2) Childrens Hospital Boston, Boston, MA; 3) Peninsula College of Medicine and Dentistry, Exeter, UK.

Adult height is a highly heritable and polygenic human trait. Using meta-analyses of genome-wide association (GWA) studies, we and others identified 43 loci that robustly associate with height variation in European populations. Remarkably, however, the associated variants at these 43 loci account for <10% of the variation in height, suggesting that many other genetic variants influence stature. To find additional height loci, we performed a larger meta-analysis, including all GWA datasets available through the GIANT Consortium (13 GWA studies; >32,000 participants). This meta-analysis identified 111 independent loci with $P<1x10^{-5}$ (~10 expected) and 50 with $P<5x10^{-7}$ (0.5 expected). More than 50 of these loci have not been previously associated with stature, indicating that the search for common variants influencing height continues to be productive as sample sizes increase. Among new interesting associations, we found an intronic SNP in *JAZF1*, a gene previously implicated in the etiology of type-2 diabetes and prostate cancer, and a SNP upstream of *KCNE2*, a potassium channel mutated in long QT syndrome and ventricular fibrillation. Pathway analysis of height associated loci revealed an enrichment of genes involved in cancer and development, and the emergence of networks that connect genes functionally. Furthermore, many of the associated loci encompass genes that are mutated in syndromes characterized by abnormal skeletal growth. Thus, our results offer an excellent list of candidate genes for human syndromes of unknown genetic etiology that have skeletal abnormalities, short stature or overgrowth as features. To help determine whether these variants also influence height in populations of African ancestry, we are testing these SNPs for association in African and African-American cohorts. In conclusion, our study of the genetic of height provides unprecedented insights into the biology of human growth and development, and also into the architecture of complex human traits and diseases.

Balloon occlusion catheter-based delivery of HDAd permits efficient hepatic transduction using clinically relevant low vector doses in nonhuman primates. *P. Ng[1], G. Stapleton[2], M. Law[2], D. Palmer[1], N. Grove[1], A. Beaudet[1], C. Mullins[2], N. Brunetti-Pierri[1]* 1) Dept Molec & Human Genetics, Baylor Col Medicine, Houston, TX; 2) Pediatric Cardiology, Baylor College of Medicine, Houston, TX.

Helper-dependent adenoviral vectors (HDAds) hold tremendous potential for liver-directed gene therapy by providing long-term transgene expression without chronic toxicity. The main obstacle hindering clinical application of these vectors is a dose-dependent acute toxicity. In humans, a systemic dose of $6x10^{11}$ vp/kg of an earlier generation Ad vector was lethal in one of two partial OTC-deficient patients whereas a ½-log lower dose ($2x10^{11}$ vp/kg) was well tolerated. Unfortunately, while safe, systemic intravenous (IV) injection of lower doses yield little to no hepatocyte transduction. Therefore, we have developed in nonhuman primates a minimally invasive method to achieve efficient hepatic transduction using low and safe HDAd doses. Briefly, a balloon catheter was percutaneously positioned in the inferior vena cava to occlude hepatic venous outflow and an HDAd expressing the baboon -fetoprotein (bAFP) reporter was injected via a hepatic artery catheter and this method was compared to simple systemic peripheral IV injection. Two baboons injected with $3x10^{10}$ vp/kg HDAd by simple systemic peripheral IV injections showed little to no transgene expression. In contrast, when the same dose of $3x10^{10}$ vp/kg was administered to two baboons using the balloon occlusion catheter approach, an increased level of transgene expression (up to 80-fold) was observed compared to peripheral IV injection. Transgene expression persisted at this high level for the duration of the observation period of at least 56 days. When an even lower dose of $1x10^{10}$ vp/kg was administered to two baboons by the balloon occlusion catheter approach, the level of transgene expression was 30-fold higher than systemic peripheral IV injection of the higher $3x10^{10}$ vp/kg dose. Thus, the balloon occlusion catheter technique permits delivery of clinically relevant low HDAd doses to achieve efficient hepatic transduction and may be a first step towards clinical application of HDAd for liver-directed gene therapy.

A Mouse Model for Hypomorphic Atr Expression. *R. L. Ragland, M. F. Arlt, T. W. Glover* Department of Human Genetics, University of Michigan, Ann Arbor, Michigan.

Both the ATM and ATR checkpoint pathways respond to DNA damage during the S/G2 phases of the cell cycle and are activated early in tumorigenesis. Unlike ATM, the investigation of ATRs role in development and tumor initiation and progression is complicated by the lethality of homozygous knock-out mice and the limited effects of heterozygous deficiency. The identification of a hypomorphic ATR mutation in the human disease Seckel syndrome-1 (SCKL1) provides a novel approach to this problem. The SCKL1 mutation leads to aberrant splicing of ATR and homozygous individuals have a 90% reduction in ATR protein expression. Here we report the creation and initial characterization of a SCKL1 mouse model. Homozygous SCKL1 mice were generated by knock-in targeting of the SCKL1 mutation, crossing to FLP transgenic mice to remove the neo cassette used for selection in ES cells, and backcrossing to C57BL/6 mice. Western blot and RT-PCR analysis established that homozygotes have no reduction in Atr protein or increase in mis-splicing as is seen in humans with SCKL1. Thus, the effects of the SCKL1 mutation differ in humans and mice. However, homozygous SCKL1 mice retaining the neo cassette have a 73-95% reduction in total Atr protein levels due to strong mis-splicing into the neo cassette, yet are viable. These mice do not show an overtly abnormal physical phenotype as of ~10 months of age, but do demonstrate aberrant Atr checkpoint activity. Under conditions of replication stress, primary fibroblasts from homozygous mice have an increase in both total gaps and breaks on metaphase chromosomes and an increase in gaps and breaks at specific common fragile sites. In addition, when compared to wild type cells, mutant cells display a significant delay in checkpoint induction as assayed by Chk1 phosphorylation. These mice have lower levels of Atr expression than heterozygote knock-out mice and have several cellular phenotypes indicative of Atr deficiency, thus providing a unique model system for studies of Atr deficiency in development and tumorigenesis.

Confirmation and Localization of Major Type 1 Diabetes MHC Telomeric Locus Near UBD. *E. Baschal, A. Steck, J. Jasinski, S. Babu, G. Eisenbarth, Type 1 Diabetes Genetics Consortium* Barbara Davis Center, University of Colorado Denver, Aurora, CO.

We believe there are additional genes, beyond HLA-DR and DQ, in the MHC region that contribute to type 1A diabetes risk, based on evidence that there is an 80% risk for the development of anti-islet autoantibodies in DR3/4 individuals who are identical-by-descent with their proband sibling. We began our search for additional genes at the furthest telomeric MHC peak of association with type 1 diabetes, near the UBD gene. We have previously reported an association with the far telomeric SNP rs1233478. We therefore analyzed the complete Type 1 Diabetes Genetics Consortium MHC panel in this region, with complete typing on 1,240 affected sib-pair families. In the additional data, rs1233478 is still highly significantly associated with type 1 diabetes (p=2.2E-24, OR=1.8). We now report an association with two additional SNPs adjacent to rs1233478, rs3131020 and rs1592410 (p=3.7E-21, OR=1.6 and p=3.0E-20, OR=1.6, respectively). These three SNPs form a haplotype, with three additional SNPs, that is present in high risk, protective, and neutral forms. The high risk haplotype (AACAAC) is transmitted to affected children 1439/2327 times (62%, p=2.1E-30). A protective haplotype (GCTGGC) is also present in our data (transmitted 1314/3054=43%, p=1.3E-14), as are neutral haplotypes. We also performed logistic regression on our data, adding the 3 SNP haplotype, HLA-DR, HLA-DQB1, HLA-B and HLA-A as explanatory variables. The final model, using forward logistic regression, contains HLA-DR, HLA-DQ, HLA-B, HLA-A, and the 3 SNP haplotype (p=8.24E-3, OR=1.3), indicating that the effect from the 3 SNP haplotype is independent of the effects of HLA-DR, HLA-DQ, HLA-B and HLA-A. The region of conservation for the high risk allele of the 3 SNP haplotype is approximately 20,000 base pairs and contains a single EST.

Shared genetics of autoimmune and inflammatory disorders: overlap of celiac disease, rheumatoid arthritis and inflammatory bowel disease. *C. C. van Diemen[1], A. Zhernakova[2], G. Trynka[1], L. Franke[1], E. A. M. Festen[1], R. K. Weersma[1], M. A. van Leeuwen[3], C. Wijmenga[1]* 1) Genetics, University of Groningen, University Medical Center Groningen, the Netherlands; 2) Medical Genetics, University Medical Center Utrecht, the Netherlands; 3) Rheumatology, University Medical Center Groningen, the Netherlands.

Recent genome-wide association studies and their follow up studies have revealed genes in autoimmune diseases (i.e. type 1 diabetes , rheumatoid arthritis, celiac disease) and inflammatory diseases (i.e. inflammatory bowel disease (IBD)) that overlap between two or more disorders, which points to a shared disease pathogenesis. In our recent celiac disease genome wide association study we have identified 8 novel loci (*RGS1*, *CCR2/CCR3*, *IL12A/Schip*, *LPP*, *TAGAP*, *SH2B3*, *IL18RAP*, *IL2/IL21*). Subsequently, we showed association of *IL2/IL21* with rheumatoid arthritis, type 1 diabetes, and IBD, and of *IL18RAP* with IBD. We have also investigated the association of the remainder of the celiac disease loci in rheumatoid arthritis and are currently analyzing them in IBD. We tested SNPs in *RGS1* (rs2816316), *CCR2/CCR3* (rs6441961), *IL12A/Schip* (rs17810546 and rs9811792), *LPP* (rs1464510), *TAGAP* (rs1738074), *SH2B3* (rs3184504), and *IL18RAP* (rs917997) in 418 rheumatoid arthritis-affected Dutch patients and 888 blood donor controls. We found no significant associations between any of the SNPs and rheumatoid arthritis (p values >0.12). The lack of association was further confirmed by imputed data from the rheumatoid arthritis dataset of the Wellcome Trust Case Control Consortium (p values >0.015). Genotyping of 518 IBD subjects is currently in progress. Thus far, this study does not provide further overlap of celiac disease genes and rheumatoid arthritis, besides the *IL2/IL21* locus. However, recent advances made in genetics of autoimmune and inflammatory disorders point to a shared genetic background for these disorders, like the association of *IL23R* with rheumatoid arthritis, celiac disease, autoimmune thyroid disease, IBD and psoriasis. This indicates that further studies are warranted using multiple inflammatory and autoimmune disorders.

Noonan syndrome and marfanoid habitus in a patient with SOS1 mutation. *M. Mathieu[1], G. Morin[1], B. Demeer[1], A. G. Le Moing[1], H. Cavé[2], C. Boileau[3], J. M. Lebrun[4], A. Verloes[2]* 1) Clinical Genetic Unit - University Hospital - Amiens - France; 2) Genetic Department - Robert Debré Hospital - Paris - France; 3) Molecular Genetic Laboratory - Ambroise Paré Hospital - Boulogne - France; 4) Service of Pediatrics - General Hospital - Soissons - France.

We report the observation of a 24-year-old male patient exhibiting features suggesting Noonan syndrome (mild mental retardation, low-set hair, hypertelorism, bilateral ptosis, dysplastic low-set ears, and webbed neck), and others compatible with Marfan disease (height at 1m83, joint hyperlaxity, arachnodactyly, pectus carinatum, severe scoliosis, myopia). Cardiac ultrasound examination was normal. Familial stature was tall (mother 1m73, father 1m83, and brothers 1m95 and 2m). Blood standard karyotype was normal. Noonan syndrome was confirmed by molecular analysis of SOS1 gene [heterozygous mutation 1654 A>C (R552G)]. However Marfan disease was not documented with a negative screening of FBN1 gene. We discuss the hypothesis of a pleiotropic effect of the SOS1 mutation, the influence of the familial stature, and the eventuality of two concomitant independent diseases.

Application of next generation sequencing technology to the identification of mouse mutations. *J. L. Moran[1], T. Fennell[1], K. Tibbetts[1], R. Levine[1], J. Wilkinson[1], K. Ardlie[1], J. Schimenti[2], M. Meisler[3], D. R. Beier[4], S. Gabriel[1]* 1) Broad Institute of MIT and Harvard, Cambridge, MA; 2) Cornell University College of Veterinary Medicine, Ithaca, NY; 3) University of Michigan Medical School, Ann Arbor, MI; 4) Brigham and Women's Hospital, Harvard Medical School.

We have established the Mouse Mutant Resequencing Initiative in order to apply high-throughput sequencing technology to the identification of mouse mutations, with the goal of expediting the recovery of biological information from existing collections of genetically mapped ethylnitrosurea-(ENU) induced and spontaneous mutants. Our first project is to sequence all exons, 5 and 3 regions in non-recombinant intervals for 11 ENU-induced mutants utilizing a pooling approach. The affected pool contains a mixture of genomic DNA from one affected animal or one heterozygous carrier for each of the 11 mutants. The control pool contains genomic DNA from the background strains for all mutants. In addition, one individual affected mutant and the corresponding control strain will be analyzed in parallel to compare sequence coverage and mutation detection in pooled vs. singleton libraries. Hybrid selection from each of the 4 libraries will be performed on Agilent oligo-arrays and resulting capture products then sequenced with the Illumina Genome Analyzer to achieve a minimum of 15X coverage per DNA sample. The total target territory for the 11 mutants is 1.78 Mb and encompasses 405 RefSeq genes and 4901 exons. Our data analysis pipeline includes identification of sequence variants within the pools and comparison of variants across affected and control pools to identify candidate single base-pair mutations. Validation of candidate mutations and assignment to individual DNAs within the pools will be performed by genotyping the observed variants in all affected and control strains individually. We anticipate that the application of next generation sequencing technologies to mouse genetics will permit rapid and cost-efficient identification of mutations in monogenic mutants as well as identification of quantitative trait loci.

POSSIBLE ROLE OF SRPX2 GENE IN BILATERAL PERISYLVIAN POLYMICROGYRIA. *S. S. Tsuneda[1], D. A. Souza-Kols[1], F. R. Torres[1], R. Secolin[1], C. V. Maurer-Morelli[1], M. M. Guerreiro[2], F. Cendes[2], I. Lopes-Cendes[1]*
1) Medical Genetics Dept., UNICAMP, Campinas SP, Brazil; 2) Neurology Dept., UNICAMP, Campinas SP, Brazil.

RATIONALE: Polymicrogyria is a malformation of cortical development characterised by multiple small gyri with abnormal cortical lamination. In bilateral perisylvian polymicrogyria (BPP), the cerebral cortex on the border and in the depth of the Sylvian fissures is thickened and abnormally infolded, more vertically oriented and, in some cases, extending more posterior up to the parietal lobes. Approximately 28 familial cases of BPP have been described, from which 75% are compatible with an X-linked heritance pattern. We have recently described a candidate locus for BPP on chromosome Xq27.2-27.3 and the SRPX2 gene is mapped in the most centromeric limits of this candidate locus. In addition, there is a report of mutations in the SRPX2 (sushi-repeat protein X-linked 2) gene in a patient with BPP and two female relatives. The objective of the present study was to perform mutation screening in the SRPX2 gene in patients form our large cohort with BPP. METHODS: Total genomic DNA obtained from 31 patients with BPP and normal controls was used as template for polymerase chain reactions (PCR) with primers for each exon sequence of the SRPX2 gene. The amplified fragments were submitted to DHPLC analysis and automated sequenced. RESULTS: DHPLC chromatography showed a different pattern of retention time in fragments of exon 1 from 2 patients belonging to different families. Sequencing analysis revealed a substitution of a guanine by a cytosine at the 30th base position of exon 1, which is predicted to be transcribed but not translated. CONCLUSION: We identified a point-mutation in the SRPX2 gene in two unrelated patients with BPP. The base-pair substitution we identified has not been reported previously and samples from control individuals are still being sequenced. In addition, the fact that the base-pair change occurred in a position that is not translated raises the possibility of a non-pathogenic mutation. Financial support: FAPESP.

The need for a well characterised UK Control Population. *B. Winney, A. Boumertit, R. Bowden, D. Davison, S. Day, E. Echeta, I. Evseeva, K. Nicodemus, S. Tonks, X. Yang, P. Donnelly, W. Bodmer* Dept. Clinical Pharmacology, University of Oxford, Oxford, OX3 7DQ, UK.

Until the recent advent of Whole Genome Association studies (WGAs), there were problems replicating significant associations between gene variation and complex diseases in studies that were generally underpowered. Population structure was widely considered to be the most significant reason. A powerful approach to this problem may be to characterise genetically both the cases and controls. Individuals from the controls can then be chosen to match the cases so as to minimise the stochastic differences between the two populations. Such a well-characterised control population would complement the current generation of WGAs. Importantly, the samples would be a resource that could be key to the search for rare variants that can be associated with disease susceptibility. We are assembling a UK control population as a resource for future studies. It will comprise 3,500 samples (3,200 collected so far), which will have been carefully selected from throughout the UK. Rural regions are targeted to avoid the admixture observed in large urban environments and volunteers are sought who were born in the same place as their parents and grandparents to ensure historical integrity. The collection will be genotyped for around 3,000 markers, with the aim of identifying about 200 ancestrally informative markers, which will then be used to match controls to cases. DNA from the samples will then be made available as a resource for future studies. An initial pilot project on about 400-500 samples, using a variety of markers, indicates that this approach is valid. MC1R data suggest structure differentiating the Celtic Fringe from Eastern England, whilst NRY data show evidence of Norse incursions into Orkney. Preliminary analyses of a larger pilot project, comprising about 700 samples and 400 markers, including HLA, provide further signals of population structure when all the samples are combined. There is also evidence of differentiation between some pairs of populations and simple admixture analyses suggest that there is an east-west gradient of Anglo-Saxon ancestry across England.

Genomic Landscapes of Pemetrexed Induced Cytotoxicity. *S. Duan[1], W. K. Bleibel[1], E. O. Kistner[2], R. S. Huang[1], S. M. Delaney[1], M. E. Dolan[1]* 1) Dept Medicine, Univ Chicago, Chicago, IL; 2) Dept Health Studies, Univ Chicago, Chicago, IL.

Pemetrexed is a novel antifolate for the treatment of mesothelioma and non-small cell lung cancer. The major toxicities of the pemetrexed-based therapy include neutropenia, leukopenia. Folate and vitamin B12 supplementation are administered clinically to reduce the side effect. In the present study, we performed a genomewide association study (GWAS) to reveal the genetic background of the pemetrexed-induced cytotoxicity using 90 human lymphoblastoid cell lines (LCLs) from the HapMap CEU population. Cell growth inhibition was evaluated following pemetrexed treatment for 72 h in media with/without additional folate-supplement. Six pemetrexed dosages (0.02, 0.1, 0.5, 1, 5 and 10 M) were evaluated in LCLs. The GWAS were performed between greater than 2 million HapMap SNPs and the area under the survival-dosage curves (AUC) after pemetrexed treatment with/without additional folate. Altogether 33 and 32 genomic regions with 87 and 87 SNPs were significantly associated with pemetrexed AUCs in the folate-supplemented and regular media, respectively (P 1×10^{-5}, FDR 0.22). Combined with the above genetic findings with the genomewide gene expression phenotypes, we identified 4 and 7 genomic regions with 23 and 25 SNPs as expression quantitative trait loci significantly associated with 22 and 21 gene expression phenotypes in the folate-supplemented and regular media, respectively (P 3.9×10^{-6}, corrected P 0.05). Significant associations between 8 SNPs and 11 gene expressions were found in both media. A general linear regression test showed 12 and 14 gene expression phenotypes significantly associated with the pemetrexed AUCs in the folate-supplemented and regular media, respectively (P 0.0012, corrected P 0.05). Multivariate linear regression analysis shows that a maximum of 68.9% and 68.6% of the overall variation for the cellular sensitivity to pemetrexed can be predicted by 6 and 8 SNPs in the folate-supplemented and regular media, respectively. The genomic landscapes of pemetrexed-induced cytotoxicity are presented through our stepwise triangular approach which can be applied in a wide range of genotype-phenotype-mapping studies.

Isolated generalized congenital anhidrosis maps to chromosome 12p11-p12. *J. Klar[1], M. Rasool[2], M. Tariq[2], A. Ali[2], I. Ahmad[2], S. M. Baig[2], N. Dahl[1]* 1) Dept Genetics & Pathology, Uppsala University, Uppsala, Sweden; 2) Health Biotechnology Division, National Institute for Biotechnology and Genetic Engineering NIBGE, Faisalabad, Pakistan.

Generalized anhidrosis (GA) is a congenital or acquired disease characterized by heat intolerance and loss of sweating, The condition is most often recognized in systemic diseases such as ectodermal dysplasia, diabetes mellitus or polyneuropathies. The pathogenesis may involve intrinsic sweat gland abnormalities or postgangliotic nerve dysfunction. Isolated congenital generalized anhidrosis (CGA) is very rare. We have identified and studied a consanguineous family of Pakistani origin with five members affected by isolated CGA. The patients suffer from severe heat intolerance without other ectodermal symptoms or any systemic disease. Skin biopsies from affected individuals revealed altered eccrine sweat gland morpholgy with absent excretory ducts and disorganized structure of secretory cells. Thermoregulatory test at 45C disclosed inability to downregulate body temperature in affected individuals when compared to controls. A genome wide autozygosity scan was initiated on the family using a 250K SNP array (Affymetrix). One large homozygous genomic region on chromosome 12p was found to be shared in all affected individuals. Microsatellite marker analysis confirmed linkage to 12p with a maximum two-point lod score of 3.42. The candidate region spans 5.8 Mb of DNA and DNA sequence analysis of candidate genes is in progress.

Molecular studies in patients with OTC deficiency. Does X-inactivation modify the genotype of heterozygtes? *L. Dvorakova[1], M. Hrebicek[1], L. Stolnaja[1], M. Bouckova[1], H. Treslova[1], H. Vlaskova[1], J. Minks[1], E. Hruba[1], O. Luksan[2], M. Jirsa[2]* 1) Inst Inherit Metabol Disorders, Charles Univ, 1st Sch Medicine, Prague 2, Czech Republic; 2) Lab. Exp. Hepatology, Inst.Clin and Exp. Medicine, Prague, Czech Republic.

Ornithine carbamoyltransferase deficiency (OTCD;OMIM 311250) is the most common inherited defect of the urea cycle. The clinical signs supported by biochemical results led to suspicion of OTCD in 28 Czech and Slovak patients, one patient came from Japan. The diagnosis was proved by mutation analysis in OTC gene (Xp21.1) in 21 patients. Five and ten male patients had neonatal and late onset form, respectively, six patients were heterozygous females. We have found 16 different mutations, 7 of them being novel. The novel mutations include c.1065AT abolishing the stop codon and c.867GA (p.K289K), which leads to a splicing defect (r.856_867del). Novel mutations p.A102P and p.S207N associated with neonatal form indicate biological importance of these amino acid residues. Among de novo mutations observed in 7 patients (4 males and 3 females), c.717+1GT (IVS7+1GT) and p.S340P were described earlier suggesting mutational hotspots in these loci. No causative mutation was found in other 4 male and 4 female patients. Haplotype analysis using intragenic polymorphisms did not support involvement of OTC gene in the family of one patient with intermitent ataxia, normal amino acids and positive allopurinol test. As liver samples of the female patients were not available, we studied X chromosome inactivation (HUMARA assay) in peripheral blood cells, urine and buccal cells of five manifesting heterozygotes and three asymptomatic carriers. X-inactivation was random in manifesting heterozygotes with null mutations and substantially skewed (75:25) in heterozygotes with missense mutations. Carriers with missense mutations and random X-inactivation were asymptomatic. Preliminary results from this small cohort of patients suggest that extreme skewing of X-inactivation is not necessary for manifestation of the OTCD phenotype in nonsense mutation carriers. Support: IGA MZ CR NR/9364-3, VZ MSM CR 0021620806 and VZ MZ CR 64165.

Protective role of a *PER3* gene polymorphism in a model of transient insomnia. *S. N. Mitkus, G. Birznieks, A. Thompson, C. A. Czeisler, C. Lavedan* Vanda Pharmaceuticals Inc., Rockville, MD.

Insomnia is the most common sleep disorder, affecting approximately 60 million Americans. Although the genetic basis of insomnia is unclear, several sleep-regulating genes have been identified. A 4-5 repeat polymorphism in a molecular clock gene, *Period 3 (PER3),* has been associated with diurnal preference and delayed sleep-phase syndrome and is thought to influence sleep structure and waking performance. We analyzed the effect of this *PER3* polymorphism on several sleep parameters, evaluated by polysomnography in healthy individuals subjected to transient insomnia. Transient insomnia was induced in 76 subjects through a 5-hour phase advance protocol and a first night effect. *PER3* 5/5 individuals had significantly greater sleep efficiency over an 8-hour sleep episode compared with non-5/5 individuals (6.2 vs 5.3 hours, p=0.023). Rate of rapid eye movement (REM) sleep accumulation was faster in 5/5 than in non-5/5 individuals (5.8 vs. 3.7 hours to accumulate 30 minutes of REM, p=0.000055), but non-REM accumulation rates did not differ. *PER3* 5/5 individuals were significantly less disrupted by induced transient insomnia than the non-5/5 individuals, suggesting that the *PER3* 5-allele protects against symptoms of insomnia induced via circadian and stress-based challenges. These results support the hypothesis that genetic variations in clock-regulating genes such as *PER3* may contribute to susceptibility to insomnia. This finding may have important implications for understanding the potential selective advantage of the *PER3* 4-5 repeat polymorphism and its role in the pathophysiology of transient insomnia.

The Johns Hopkins Center for Hypotonia: New Multidisciplinary Clinic for Diagnosis and Support of Individuals with Hypotonia. *E. Lisi, R. Cohn* Inst Genetic Medicine, Johns Hopkins Univ, Baltimore, MD.

Hypotonia is defined as diminished tone of skeletal muscle in conjunction with decreased resistance of muscles to passive stretching. It is associated with over 500 disorders, though many hypotonic patients have no underlying diagnosis for their condition. The Johns Hopkins Center for Hypotonia was started in 2007 to focus on identifying, supporting, and treating patients with conditions associated with hypotonia. Through May, 2008 a total of 108 patients were seen during 24 clinic days with varying degrees of hypotonia with or without other anomalies. Most patients had seen several other physicians prior to their referral. Fourteen patients came with established diagnoses for multidisciplinary care. Of the remaining 79 patients, a total of 29 new diagnoses were made (31%), including chromosomal abnormalities, syndromic diagnoses such as Rett, Angelman Joubert, Cohen, Greig cephalopolysyndactyly, Bannayan-Riley-Ruvelcaba, and Madelung disease. In addition,we diagnosed several forms of muscular dystrophies, metabolic myopathies, mitochondrial and connective tissue disorders. We have also identified a cohort of nine patients with hypotonia, macrocephaly and variable degrees of developmental delay. We are currently in the process collecting more patients to elucidate the molecular basis for this association using SNP genotyping. In summary, the ultimate goal of our center is to combine clinical and basic science approaches to gain a deeper understanding of the causes and natural history of hypotonia. We hope to use these insights to develop novel therapeutic strategies to help the individual patient to achieve their maximum cognitive and physical performance.

Two intragenic NIPBL deletions and one contiguous gene syndrome detected by MLPA in CdLS patients. *S. Russo[1], C. Gervasini[2], M. Masciadri[1], P. Castronovo[2], D. Milani[3], A. Cereda[3], G. Zampino[4], A. Selicorni[3], L. Larizza[1,2]* 1) Molecular Genetics Institute Auxologico Italiano Milan Italy; 2) Medical Genetics San Paolo School of Medicine University of Milan Italy; 3) Pediatric Dep Fondazione Ospedale Maggiore Policlinico Mangiagalli Regina Elena Milan Italy; 4) Department of Pediatrics Cattolica University Rome.

Cornelia de Lange syndrome (CdLS) is a rare, multiple congenital anomaly/mental retardation disease, characterized by facial dysmorphisms, growth deficiency, psychomotor delay and malformations of the upper limbs. About half of CdLS patients are mutated in the NIPBL gene (5p13.2) encoding for a member of the adherins family, involved in the processes of chromatid cohesion and enhancer-promoter communication. Mutations in the SMC1L1 and SMC3 genes (two subunits of the cohesin complex) responsible for an X-linked and another autosomal form respectively are thought to contribute to up 5% of all CdLS cases. We screened for NIPBL and SMC1L1 mutations the first Italian cohort of CdLS patients which has been evaluated by the recently approved diagnostic system and a global score (Selicorni et al. 2007). By this analysis besides detecting NIPBL and SMC1L1 mutations we sorted out a platform of CdLS patients to be further screened by other approaches. Here we report on a refinement of NIPBL mutation scan by MLPA kit. Analysis of 50 pts allowed us to detect three partial deletions of NIPBL gene: two encompassing exon 2 and exon32, and the third affecting exons 1-10. Segregation analysis by DNA polymorphic markers showed that the latter deletion also involves sequences upstream NIPBL. By refined FISH characterization the deletion turned out to extend 2Mb from AGXT2 gene to NIPBL IVS10. It includes 14 genes apart NIPBL, thus featuring a contiguous gene syndrome associated with an extremely severe phenotype of the patient. A mild phenotype was observed in the pt carrying exon 2 deletion and a severe clinical presentation is present in pt carrying exon 32 deletion. Application of MLPA to CdLS pts found negative to standard mutation screening is an adjunct tool to disclose full NIPBL mutation spectrum and address genotype-phenotype correlations.

NRAMP1 Polymorphisms and Susceptibility to Tuberculosis in Turkish Population. *A. Y. EKMEKCI[1], F. Ozkinay[1], H. Onay[1], F. Bacakoglu[1], A. Sayiner[1], S. Z. Guclu[2], S. Pehlivan[3], O. Cogulu[1], A. Aykut[1], C. Gunduz[1], C. Ozkinay[1]* 1) Ege University, Faculty of Medicine, Turkey; 2) Suat Seren Chest Diseases and Thoracic Surgery Training Hospital, Turkey; 3) Gaziantep University, Faculty Of Medicine, Turkey.

Tuberculosis(TB) is the most common mycobacterial disease that is caused by Mycobacterium tuberculosis (M. tuberculosis). Twin studies in different ethnic populations have suggested that host genetic factors are as effective as environmental factors in susceptibility to tuberculosis disease. NRAMP1 protein localises on macrophage membrane and regulates macrophage functions against M. tuberculosis and other intracellular microorganisms. In NRAMP1 gene, $5(CA)_n$ is a functional repeat polymorphism and controls the NRAMP1 expression in a luciferase reporter system. INT4 (469+14G C) polymorphism is a single base change in intron 4. 3UTR (1729+55del4) polymorphism is 4 bp deletion in the 3untranslated region and D543N polymorphism is a G to A base change in exon 15. In our study we aimed to investigate the association between the four polymorphisms and compound genotype frequencies of those polymorphisms of the NRAMP1 gene, and susceptibility to TB including clinical severity of disease in different age groups. Four hundred and seventy two HIV negative Turkish TB patients (340 males, 132 females) and 571 age-matched healthy controls(432 males, 139 females) were included in the study. Mean ages of the patient and control groups were 39,21 18.597, 36,48 13,963 respectively. All TB patients were divided subgroups as pediatric TB patients who were younger than 18 years of age and as adult TB patients who were older than 18 years of age (n: 57, n:415, respectively). Our results indicate that none of the NRAMP1 polymorphisms investigated are associated with the disease susceptibility but they might effect the age of onset, clinical variations and the progression of the disease.

Association of *IL2/IL21* variants with ulcerative colitis. *E. A. M. Festen[1,2], A. Zhernakova[3], C. Wijmenga[1,3], R. K. Weersma[2]* 1) Genetics, University Medical Center Groningen, University of Groningen, Groningen, the Netherlands; 2) Gastroenterology and Hepatology, University Medical Center Groningen, Groningen, the Netherlands; 3) Medical Genetics, University Medical Center Utrecht, Utrecht, the Netherlands.

Both celiac disease and inflammatory bowel diseases (IBD) are characterized by inflammation of the intestinal mucosa and dysregulation of the immune system. IBD consists of ulcerative colitis (UC) and Crohns disease (CD). Previous studies have identified shared susceptibility genes for both IBD and celiac disease. Genetic variants in a region harbouring *IL2* and *IL21* have been found to be associated with celiac disease susceptibility in a genomewide association study performed by van Heel et al (Nat Genetics 2007;39:827-9). *IL2-/-* mice develop a disease that strongly resembles UC. Furthermore IL21 has been proven to be produced in excess in the inflamed mucosa of IBD patients. We hypothesized that the *IL2/IL21* region is a shared susceptibility gene region for both IBD and celiac disease and performed a case-control study to look for an association between variants in *IL2/IL21* with IBD. The study population consisted of 518 Dutch IBD patients (311 CD and 207 UC) and 1422 healthy UK controls. We analyzed the strongest celiac disease associated SNPs, namely rs6822844, rs13151961 and rs6840978, by Taqman genotyping. SNP rs13151961 showed a protective effect of allele G with UC (p = 0.035) with an OR of 0.73 (CI 1,02 to 1,86). Allele T of rs6822844 was also found to have a protective effect for UC (p = 0.033) with an OR of 0.72 (CI 1.03 to 1.88). Allele C of rs6840978 was found more frequently in UC patients (82.5%) compared to normal controls (78.1%), although this association was not significant. None of the SNPs tested showed any association with CD. CONCLUSIONS: We have shown that genetic variants in the *IL2/IL21* region are associated with UC. We found a protective effect for UC of allele G of rs13151961 and a protective effect for UC of allele T of rs6822844. These findings confirm that *IL2/IL21* is a susceptibility gene for both celiac disease and UC and suggests a shared common pathogenesis for these diseases.

Familial clustering of superior orbicularis oris muscle defects in families with cleft lip and cleft palate. *C. M. Klotz, M. E. Cooper, T. McHenry, K. Neiswanger, M. L. Marazita* Center for Craniofacial and Dental Genetics, University of Pittsburgh, Pittsburgh, PA.

Cleft lip (CL) with or without cleft palate (CP) is common, with average birth prevalence of 1 per 1000 live births. CL/P phenotypes range from overt clefts to minimal microforms. Occult defects of the orbicularis oris (OO) muscle appear to be part of this spectrum (Neiswanger et al, 2007). The purpose of the current study is to investigate familial clustering of OO defects in families with a history of CL versus CP. Breaks in the continuity of the OO muscle visualized on ultrasound were scored as affected. All participants were placed into one of five family groups: CL = all affecteds in the family have CL only; CP = all have CP only, CLP = all have CL+CP; CL/CLP = at least one affected has CL and at least one has CL+CP, CP/CLP = at least one has CP and at least one has CL or CL+CP. Two larger groups were also assigned: a cleft lip group, including CL, CLP, and CL/CLP, and a cleft palate group, including CP and CP/CLP. 1101 unaffected family members were included in these analyses, of whom 76 (6.9%) had OO defects. By family group: CL - 6/106 (5.7%), CP - 0/27 (0.0%), CLP - 41/501 (8.2%), CL/CLP - 26/407 (6.4%) and CP/ CLP - 3/60 (5.0%). Fishers exact tests comparing the percent OO defects across all groups resulted in a p-value of 0.51. Similarly, the cleft lip group (73/1014, 7.2%) compared to the cleft palate group (3/87, 3.4%), resulted in a p-value of 0.27. Notably, there is an increase in OO defects seen in the unaffected relatives from cleft lip families versus cleft palate families. However, the difference is not statistically significant and will require larger sample sizes to investigate further. If the difference is confirmed, there will be implications for recurrence risk estimation in families with different cleft types. NIH grants: R01-DE016148, R21-DE016930, P50-DE016215, R37-DE08559.

SLC30A8 Variant Is Associated With Muscle Size and Response To Resistance Training. *F. Orkunoglu-Suer[1], J. M. Devaney[1], R. Patel[2], K. Adham[2], J. Larkin[2], H. Gordish-Dressman[1], C. C. Brandoli[1], L. L. Tosi[1], E. P. Hoffman[1], FAMUSS study group* 1) Res Ctr Gen Med, Children's Nat Med Ctr, Washington, DC; 2) George Washington University School of Medicine, Washington DC.

Objective: SLC30A8 encodes the protein zinc transporter-8 (ZnT8), which is expressed mainly in the islets of Langerhans of the pancreas. Zinc deficiencies and transport problems have been associated with Type II diabetes. Exercise improves glucose metabolism and has been demonstrated to help reduce the negative impact of these disorders. We hypothesized that the single nucleotide polymorphism (SNP) rs13266634 (C37447T; R325W) of the SLC30A8 gene would be associated with an individuals measures of physical fitness and response to exercise. Methods: Healthy young adults (n = 626) enrolled in the FAMUSS study were placed on a scheduled resistance-training program for 12 wks. Baseline and post training measurements of muscle volume, subcutaneous fat volume, baseline strength as measured by one-repetition max (1RM) and triglyceride levels were ascertained. DNA samples were genotyped using Taqman assays. A semi-automated MRI analysis tool, RAPIDIA, was used to calculate humeral muscle volume before and after training. Analysis of covariance (ANOVA) was used to identify statistically significant differences in measures of muscle and fat volume, strength, and triglyceride levels among genotypes.Results: Female participants (n = 323) with two copies of the T allele were more likely to gain muscle volume ($p = 0.03$) and lose fat ($p = 0.003$) following the resistance-training program. Males (n = 303) with two copies of the T allele had higher 1RM strength ($p = 0.04$), higher baseline whole muscle volume ($p = 0.002$) and higher triglyceride values ($p = 0.006$).Conclusions: The T allele of rs13266634 is associated with greater muscle hypertrophy in response to exercise training in females and increased baseline strength/muscle volume in males. Our results suggest that this SNP may play a protective role against type II diabetes.

Chromosome 5q14.3-q21 deletion in a patient with overlapping features of Oculo-Oto-Dental Syndrome. *M. F. Walsh[1], N. L. Sobreira[1], E. Lisi[1], D. A. S. Batista[2,3], T. Wang[1]* 1) Institute of Genetic Medicine; 2) Pathology Department, Johns Hopkins University; 3) Kennedy Krieger Institute, Baltimore MD.

The association of bilateral high-frequency sensorineural hearing loss, dental anomalies, and iris coloboma was described previously in one British family as oculo-oto-dental syndrome (OOD). Autosomal dominant inheritance with variable expressivity was implicated. A 12-cM critical region for OOD was initially identified at 20q13.1 by linkage study (Vieira et al., 2002). A recent genome-wide scan in this family and two others with otodental syndrome (OD, MIM166750) identified a shared 43 kb deletion involving FGF3 at 11q13 in all three and a larger deletion involving FADD in the family with OOD (Gregory-Evans et al, 2007). Here we describe an eleven-year old male with bilateral iris coloboma with small optic nerves, duplicate rows of frontal incisors, and high frequency hearing loss. Additional features include brachydactyly, a shawl scrotum, moderate intellectual disability, attention deficit disorder, aggressive and stereotyped behaviors. Family history was negative. Consanguinity and prenatal exposure were denied. CT and ultrasound studies revealed normal ossicles in the middle ear and renal structure. A high-resolution karyotype and BAC-microarray CGH with 4200 clones (BlueGnome) identified a genomic deletion 5q14.3-q21.1 that was confirmed by FISH. The size of the deletion estimated from the array CGH is between 7.3 to 8.9 Mb including 23 annotated genes. None of these genes is known to cause hearing loss, dental or iris anomaly. Outside this deletion on 5q, there are 26 genes related with hearing loss, one with iris coloboma, and one with supernumerary teeth. Clinical phenotype in this patient overlaps with key features of OOD syndrome but also includes other malformations, intellectual disability, and behavioral issues. This may represent a contiguous gene syndrome suggesting locus heterogeneity for OOD-like phenotype. Study of the breakpoint and expression profile of genes that may be affected will help to understand genetic basis for OOD-like phenotype and identify novel genes that are important to normal hearing, ocular, and dental development in humans.

Genome-wide scan identified a copy number variation in VPS13B associated with bone strength. *L. Zhao[1], T. B. Jin[2], Y. F. Pei[3], X. G. Liu[3], B. Y. Sha[1,4], Y. Z. Liu[2], Y. J. Liu[2], R. R. Recker[1], H. W. Deng[2,3,4]* 1) Osteoporosis Res Ctr, Creighton Univ Med Ctr, Omaha, NE; 2) School of Medicine, University of Missouri - Kansas City, Kansas City, MO; 3) Xi'an Jiaotong University, Xi'an, P.R.China; 4) Hunan Normal University, Hunan, P R China.

Background: DNA copy number variation (CNV) is a rich and important source of genetic diversity for human diseases. Hip fracture is a severe and common injury in the elderly. The major risk factor of hip fracture is reduced femoral bone strength, which is under strong genetic control and the genetic factors are largely unknown. Materials and Methods: We conducted a genome-wide CNV analyses for femoral neck bone strength in 985 unrelated subjects using Affymetrix 500K array set. Femoral neck bone mineral density (BMD) was measured by dual energy X-ray absorptiometry (DXA). Femoral neck cortical thickness (CT), cross-sectional area (CSA), and buckling ratio-an index for bone structural stability, were estimated using DXA-derived femoral neck BMD and bone area. Results: A CNV located in the VPS13B (vacuolar protein sorting 13 homolog B (yeast)) gene is significantly associated with femoral neck BMD (P= 0.0001), CT (P=0.0001), BR (P=0.017), and CSA (P=0.003) in the whole sample. The association results for femoral neck BMD and CT remain significant even after conservative Bonferroni correction (P=0.026 for femoral neck BMD and P=0.027 for CT). Significant results (P<0.05) are also found for all the bone strength phenotypes in both male and female subgroups. We further compared raw bone strength values between subjects with one copy number and two copy numbers of the CNV. Comparing to the normal subjects, those with one copy of the CNV have higher femoral neck BMD (P = 0.003), higher CT (P = 0.004), lower BR (P = 0.02), and higher CSA (P = 0.05), which consistently leads to good bone strength. Conclusions: This is the first report of a genome-wide CNV association for femoral bone strength. Our results suggest that VPS13B gene is important for femoral bone strength.

Analysis of telomeres in peripheral blood mononuclear cells from patients with Diamond-Blackfan Anemia. *I. Dianzani[1], E. Pavesi[1], F. Avondo[1], A. Aspesi[1], E. Garelli[2], P. Quarello[2], F. Campagnoli[2], A. Carando[2], C. Dufour[3], U. Ramenghi[1]* 1) Dept Med Sci, Univ Piemonte Orientale, Novara, Italy; 2) Hematology Unit, Dpt.Pediatrics, Univ.Torino; 3) G. Gaslini Childrens Hospital, Genova.

Diamond Blackfan Anemia (DBA, MIM 105650) is an autosomal dominant erythroid hypoplasia in which erythroid progenitors do not differentiate efficiently and are susceptible to apoptosis. All known genes mutated in DBA encode proteins of the 40S and 60S ribosomal subunits. Since RPS19, mutated in 25% of DBA patients, is required for the maturation of the 40S ribosomal subunit, the erythroid hypoplasia observed in DBA may be the result of a defect in ribosome biogenesis. However, an explanation for the tissue specific clinical manifestations is still elusive. Other hypotheses suggest that DBA may be due to loss of an alternative function of the mutated RP. In a proteomic study, we identified 159 proteins associated with RPS19 in a pull-down assay. Included among this group were components of the H/ACA small nucleolar Ribonucleoprotein Particles (H/ACA snoRNP) complex: dyskerin, NOLA1 and NOLA3. This complex, which also includes NOLA2 and specific H/ACA RNAs, is required for three basic cellular functions, ribosome biogenesis, pre-mRNA splicing and telomere maintenance. Several components of this complex have been identified as mutated in another bone marrow failure syndrome, dyskeratosis congenita (DC). It appears that the defect in telomere maintenance is primarily responsible for the clinical features of DC. Our study linked RPS19 to another possible function implicated in bone marrow failure. To ascertain whether a defect in telomere maintenance was present in DBA patient cells, we have evaluated telomere length in DNA obtained from peripheral blood mononuclear cells of 46 DBA patients (16 mutated in RPS19) and 93 age-matched controls using a real-time quantitative PCR method [Cawthon Nucl Acids Res 2002]. Only one patient showed a defect in telomere length and may be affected by an atypical form of DC. Our data agree with the study of Alter et al. [Blood 2007], performed on 14 DBA patients not characterised for mutations. Telomere maintenance does not seem involved in the pathogenesis of DBA.

The Allelic Architecture of Common Diseases and Its Consequences. *C. C. A. Spencer, E. Hechter, P. Donnelly* The Wellcome Trust Centre for Human Genetics, University of Oxford, Roosevelt Road, Oxford, OX3 7BN.

Genome-wide association studies have been extremely successful in finding loci associated with common complex human diseases. Where only a handful of such loci had been reliably established a few years ago, there are now around 100 across more than 20 diseases. For the first time we are beginning to gain real insights into the allelic architecture of common diseases. However our view is currently distorted and incomplete: for the majority of diseases there remain many more loci to be found and, of those loci already identified, we are yet to pinpoint the causal variants. In most cases, when we see a signal in an association study it will arise at SNPs which are different from, but correlated with, true causal variants. Effect sizes estimated at the SNPs discovered from association studies will thus tend to be smaller than the true effect sizes. We study this issue, and show that true effect sizes could be substantially larger than those being reported. We describe a Bayesian approach to assess probabilistically the true distribution of genetic effects for common diseases. Our results have important implications for fine-mapping, future study design and choice of genotyping chip and for assessments of genetic risk in a population context.

Efficiency of CGH-Array in detecting chromosomal mosaicism: a case of Tetrasomy 12p. *N. Marle[1], P. Callier[1], AL. Mosca[1], C. Thauvin[2], A. Masurel[2], N. Madinier[3], C. Bonnet[4], L. Faivre[2], F. Mugneret[1]* 1) Laboratoire de Cytogénétique, CHU Le Bocage, Dijon, France; 2) Département de Génétique Clinique, Hôpital dEnfant, Dijon, France; 3) Clinique Sainte-Marthe, Dijon, France; 4) Unité de Cardiologie Pédiatrique, Hôpital dEnfants, CHU Dijon, France.

Array-CGH provides a higher level of genomic analysis over the standard chromosome analysis. Therefore, it has become a powerful tool in the screening of patients presenting with idiopathic mental retardation. Less expected is the ability of array-CGH to detect low-level chromosomal mosaicism in peripheral blood, as mosaicism for a supernumerary metacentric isochromosome 12p resulting in Pallister-Killian syndrome. Here we report on the first child of healthy non consanguineous parents. He was born at 36 weeks of gestation, after a normal pregnancy. Birth weight, length and head circumference were respectively 3440 g, 46 cm and 33.5 cm. Clinical examination revealed a short forehead, short webbed neck, short ears, fissures of the auricular lobes and multiple pits, unique palmar crease, micropenis and umbilical hernia. Renal and cardiac ultrasound showed enlarged kidneys and atrial septal defect, cerebral MRI revealed partial agenesis of corpus callosum. At last Postnatal cytogenetic analysis on peripheral lymphocytes with RHG and GTG banding revealed a 46,XY chromosome pattern. BAC-array (Integragen, 4898 clones) was performed on DNA extracted from peripheral blood sample. It showed a gain of the entire short arm of chromosome 12. FISH analysis with CEP12 probe on peripheral blood culture was normal in 200 cells examined. Chromosome analysis on culture fibroblasts from a skin biopsy showed a 47,XY,+i(12)(p10)[24]/46,XY[1] karyotype. FISH analysis with CEP12 probe revealed a 90% mosaic 12p tetrasomy on culture fibroblasts and a 1% mosaic 12p tetrasomy on a second peripheral blood sample. As expected, parental karyotypes were normal. Here we report the third case of Pallister-Killian (second case resulting from a supernumerary isochromosome 12p) identified using array-CGH. Based on published data, we discuss the usefulness of array-CGH in the detection of low-level chromosomal mosaicism.

Mutation analysis of the *CREBBP* gene in a cohort of Rubinstein-Taybi syndrome patients. *M. Losekoot[1], C. A. C. van der Lans[1], D. J. M. Peters[2], M. J. van Belzen[1]* 1) Clinical Genetics, Laboratory for Diagnostic Genome Analysis (LDGA), Leiden University Medical Center, Leiden, The Netherlands; 2) Human Genetics, Leiden University Medical Center, Leiden, The Netherlands.

Rubinstein-Taybi syndrome (RSTS) is a rare disorder affecting approximately 1/100,000 newborns. The syndrome is characterized by mental and growth retardation and a particular dysmorphology mainly concerning the face, hands and feet. The most frequent cause of RSTS are mutations in the *CREBBP* gene, which are found in 30-50% of patients. Furthermore, microdeletions involving the *CREBBP* gene have been found in ~10% of patients and a further 1-3% can be explained by mutations in *EP300*. Mutations in *CREBBP* are primarily nonsense, frameshift and splice site mutations that occur over the entire coding region, although also several pathogenic missense mutations have been identified. These are predominantly located in the conserved, functional regions of the gene. We tested a cohort of 30 patients suspected of having RSTS for mutations in the *CREBBP* gene and detected 11 pathogenic mutations (37%). In addition, 8 variants of unknown clinical significance were found, of which 3 are likely to be pathogenic, leading to an overall mutation detection rate of ~42%. Therefore, sequence analysis of *CREBBP* is an important part in the diagnostics of RSTS. Mutation analysis by direct sequencing of the entire coding region of *CREBBP* is now available as a diagnostic service at the LDGA laboratory. This service is available both in and outside the Netherlands (see also our website www.lumc.nl/4080/). Recently, MLPA analysis of all exons of the *CREBBP* gene has been added to our diagnostic package for RSTS (data not available yet).

Genome-wide mapping of protein microarray-generated pollen allergy phenotypes in a founder population. *K. Vigh[1], D. Preuss[2], C. Ober[1]* 1) Dept Human Genetics, Univ Chicago, Chicago, IL; 2) Dept Molecular Genetics and Cell Biology, Univ Chicago, IL.

Mapping genes for complex diseases, such as allergy, that are influenced by many genes and strong environmental factors remains challenging. The increasing accessibility of high-resolution genotyping has aided these studies. However high-resolution phenotyping, which is also integral to successful genetic mapping, is still an obstacle for many traits. Here, we studied quantitative, intermediate allergy phenotypes in the Hutterites, a young founder population who live a communal lifestyle. We developed a protein microarray to measure allergen-specific IgE (AS-IgE) to a multitude of allergens, and screened serum from 564 Hutterites. We estimated the heritability of 38 traits (AS-IgE to 38 allergens) and found that 18 were significantly heritable with narrow heritabilities (h^2) ranging from 0.18-0.41 and broad heritabilities (H^2) ranging from 0.46-0.71. AS-IgE to groups of allergens, such as all grasses ($p=5.96x10^{-6}$), all trees ($p=6.07x10^{-5}$), and all pollens ($p=5.11x10^{-6}$) were the most heritable phenotypes. We, therefore, performed genome-wide linkage and association mapping with these 3 traits and 1520 markers comprising microsatellites and SNPs. Significant linkage was seen for all 3 traits in a region on chromosome 22q11 that contains several genes, including the *XKR3*, *IL17RA*, and *CERCR-1*, *-5*, *-6* (minimum $p=1.47x10^{-5}$; genome-wide p=0.007 determined by 1000 permutations). Evidence of suggestive linkage for all 3 traits was observed on chromosome 9p21 with a SNP in the single exon of the class I interferon (*IFNB*) (minimum $p=4.68x10^{-4}$) and with two microsatellites (minimum $p=3.09x10^{-4}$) flanking the *PLAA* and *TEK* genes. Association mapping showed that AS-IgE to all trees (locus $p=1.11x10^{-7}$; genome-wide $p<0.001$) and all pollens (locus $p=2.44x10^{-6}$; genome-wide p=0.004) was associated with a SNP in the *EIF2AK2* gene on chromosome 2p22, which is induced by class I interferons. These results indicate that variation in genes on chromosomes 2p22, 9p21, and 22q11 contribute to multiple allergy phenotypes assayed by our array, with possible interaction between some of the genes.

Analysis of Tetrahydrobiopterin Associated Genes in Autism. *N. Schnetz-Boutaud[1], K. Brown[1], K. Gainer-Luci[1], M. L. Cuccaro[2], J. R. Gilbert[2], H. H. Wright[3], R. K. Abramson[3], M. A. Pericak-Vance[2], J. L. Haines[1]* 1) Center for Human Genetics Research, Vanderbilt Univ. Medical Center, Nashville, TN; 2) Miami Institute for Human Genomics, Univ. Miami, Miami, Fl; 3) W.S. Hall Psychiatric Institute, University of South Carolina, Columbia, SC.

Autism, Aspergers Syndrome, and Pervasive Developmental Disorders (PDD-NOS) are all classified as Autism Spectrum Disorders (ASDs). ASDs are neurodevelopmental and neurobehavioral disorders with an onset early in childhood and a preponderance of affected males (~4:1). The ASDs are characterized by social communication impairments and the presence of repetitive and stereotypic behaviors. ASDs have a strong genetic component, suggested by numerous different measures including the high concordance rates for MZ twins (60%-90%) compared to DZ twins (0%-10%). However, the genetic architecture of ASDs has so far eluded explanation. Disturbances in the dopamine and serotonin pathways have been consistently reported in ASDs; genetic analyses of many of the genes underlying these pathways have not yielded strong associations with ASDs. Tetrahydrobiopterin (BH4) is the essential cofactor in the synthesis of both dopamine and serotonin. We therefore genotyped 25 SNPs in 9 genes (SPR, QDPR, PCBD1, PTS, GCH1, GCHFR, PAH, TH, and TPH2) of the BH4 pathway in a total of 403 families. The most significant result was in the PTS (6-@pyruvoyltetrahydropterin synthase) gene on chromosome 11q22 (p=0.003, pedigree disequilibrium test), but this did not survive correction for multiple comparisons (p=0.07). Examining the male only subset (n=303), or testing for 2- or 3-way interactions did not reveal any further association. We thus conclude that variations in genes related to tetrahydrobiopterin are not strong risk factors for autism.

Profiles of structural variantion between ethnic groups in human populations. *L. Bassaganyas[1,2], M. García-Aragones[1], M. Montfort[2], G. Escaramís[2], M. Cáceres[1], L. Armengol[1,2], X. Estivill[1,2,3]* 1) Genes and Disease Program, Center for Genomic Regulation; 2) Public Health and Epidemiology Network Biomedical Research Center (CIBERESP); 3) Pompeu Fabra University, Barcelona.

Genetic variation contributes to common phenotypic differences between individuals, populations and species. It is also a key element for disease predisposition and is an evident potential substrate for natural selection. Recently, the use of genome-wide molecular methods such as array-based comparative genome hybridization (aCGH) and comparative genome intensities (aCGI), have revealed the existence of an unexpected amount of copy number variants (CNVs), genomic segments ranging in size from one kilobase to several megabases present at variable copy number in comparison with a reference genome. The aims of our study were to determine the existence of population-specific genomic copy number variation and to identify genes located in these regions that might contribute to phenotypic differences, as well as to differential susceptibility to common disease and environmental exposures of human populations. We have selected 343 individuals from eleven human populations of diverse geographic ancestry from the Human Genome Diversity Panel and 86 individuals from two populations of the HapMap collection. To detect population-specific CNVs we have used aCGH (H244K Agilent) and have identified a total of 199 loci that fulfilled our inclusion criteria. 124 of these regions were already described in the Database of Genomic Variants, and 153 genes were totally or partially overlapping the identified loci. Interestingly, in this set there is a significant enrichment of genes involved in neurophysiological functions, metabolic activity, immune response and sensory perception, and mainly, encode for membrane proteins. We propose that the CNVs detected in this study could explain, at least in part, differences in disease predisposition among individuals from different populations and could provide important clues on the recent adaptation of humans to different environments.

An apparent paradox of prevalence of Metabolic Syndrome by different definitions and its reconciliation. *B. M. Chakraborty, S. M. Pinney, W. Niu, R. Chakraborty* Dept Environmental Health, Univ Cincinnati Col Medicine, Cincinnati, OH.

Abdominal obesity, high blood pressure, high blood sugar, high triglycerides, and low high density lipoprotein often occur together in individuals throughout the world. Joint occurrence of several of these conditions is known as the Metabolic Syndrome (MS). Since the components of MS are risk factors of cardiovascular diseases, diabetes, and hypertension, MS has been studied in relation to morbidity/mortality of these diseases. Several definitions of MS have been suggested which vary by the components that must be included in MS and their cut-off values. Consequently, prevalence of MS by different definitions is not readily comparable, making inter-study comparisons difficult. We studied differences of prevalence of MS by two definitions (ATPIII, suggested by the NIH National Cholesterol Education Program Panel of 2001; and IDF, prescribed by the International Diabetes Federation in 2003) in a cohort of the Fernald Medical Monitoring Program (FMMP) of Ohio, USA. From physical examination data of 2005-2006 on 2,985 Caucasian subjects (1,232 men and 1,753 women) of age 30 to 94 years, we estimated crude, age-specific and age-adjusted rates of MS by both ATPIII and IDF criteria. While a substantial proportion of individuals in this cohort has MS by both definitions (30.5% of total, 33.8% of men, and 28.2% of women), in both genders the IDF-definition yields a higher prevalence compared with that by ATPIII (48.1% vs. 36.1% in men, and 36.7% vs. 28.2% in women) due to the more inclusive criteria of abdominal obesity and glucose abnormality in the IDF-definition. Apparent paradox arises in the observation that a small but significant number of individuals (1.4% of total, 2.4% of men and 0.7% of women) have MS by the ATPIII criteria, but not by that of IDF. The IDF requirement that all MS must have abdominal obesity (with 2 or more of the other components), but by ATPIII any 3 or more defines MS explains this observation. With increasing trend of MS by age, the gender difference (MS more prevalent in men than women) and increased rate of MS by IDF holds true for age-specific and age-adjusted rates.

The neuronal nitric oxide synthase gene (*NOS1*) contributes to increased risk of stroke. *H. Manso[1,2], T. Krug[1,3], J. Sobral[1,2], B. V. Fonseca[1,3], I. Albergaria[2], G. Gaspar[2], J. M. Ferro[3], S. A. Oliveira[1,3], A. M. Vicente[1,2], PORTuguese Stroke GENetics (PORTSGEN) Group* 1) Inst Gulbenkian de Ciencia, Oeiras, Portugal; 2) Inst Nacional de Saúde Dr. Ricardo Jorge, Lisboa, Portugal; 3) Inst Medicina Molecular, Lisboa, Portugal.

Stroke is a complex disease resulting from the interplay between genetic and well-established environmental risk factors. Despite the existence of drugs that control the latter, it has not been possible to reduce the incidence of stroke in developed countries, reinforcing the importance of genetics in stroke risk. Several lines of evidence suggest that carotid atherosclerosis may contribute to ischemic stroke risk, as atherosclerotic plaques are vulnerable to mechanical fissure, leading to thrombus formation and stroke. Nitric oxide (NO) is known to have several vasoprotective or anti-atherosclerotic properties. This, together with the finding that the neuronal nitric oxide synthase (nNOS/NOS1) protein and mRNA are detected in early and advanced atherosclerotic lesions but not in normal tissue, suggests that the *NOS1* may contribute to stroke risk. 31 *tag* SNPs covering genetic variation in coding and flanking regions of *NOS1* were tested for association with stroke risk in a Portuguese sample of 566 ischemic patients and 530 controls. Logistic regression analysis was carried out to adjust for significant stroke risk factors age, sex, smoking, hypertension and diabetes. 11 *NOS1* SNPs were associated with stroke risk (0.0003*P*0.0483); 2 SNPs remained significant after Bonferroni correction for multiple testing. In addition, 10 haplotypes containing at least one of the associated SNPs, and 3 other haplotypes were associated with stroke (0.0007*P*0.0483). Given the well documented neuroprotective properties of nNOS after stroke, we tested *NOS1* SNPs for association with stroke functional recovery, assessed using the modified Rankin Scale (mRS) at 3 months in 430 ischemic stroke patients (good recovery: mRS1), adjusting for clinical severity parameters. No significant association was found with stroke recovery. These results indicate that *NOS1* gene variants influence the risk of ischemic stroke, but not functional recovery.

A graphical model approach for inferring large-scale networks integrating gene expression and genetic polymorphism. *J. Chu, S. T. Weiss, V. J. Carey, B. A. Raby* Channing Laboratory, Brigham and Women's Hospital, Harvard Medical School, Boston, MA.

Graphical models (e.g. Bayesian networks) have been used frequently to describe complex interaction patterns and dependent structures among genes and other phenotypes. Estimation of such networks has been a challenging problem when the genes considered greatly outnumber the samples, and the situation is exacerbated when one wishes to consider the impact of polymorphisms in genes. Here we describe a multi-step approach to infer a gene-SNP network from gene expression and genotyped SNP data. Our approach is based on 1) identification of candidate genes from previous integrative genomic analysis. 2) construction of a graphical Gaussian model (GGM) based on estimation of partial correlations among all genes and FDR multiple testing. 3) extraction of a sub-network of genes directly linked to the candidate genes based on the GGM in step 2. 4) Cis-association SNPs within 200Kb of the genes in the sub-network added to complete the network. We demonstrate the method by building a complex gene-SNP network around Interleukin 1B (IL1B) -- a biologic candidate in asthma pathogenesis. The model was built using 534,290 genotyped SNPs (Illumina Infinium 550K array) and gene expression data on 22,177 genes from total RNA derived from peripheral blood CD4+ lymphocytes in 154 asthmatics. Significant gene-gene and gene-SNP associations have been identified based not just on their co-expression but through whole network modeling. Specifically, GGM identified 271 genes demonstrating direct association with IL1B (posterior probability >0.5). The model revealed evidence of cis-acting genetic variants for 26 of these genes that also manifested evidence of trans-effects on IL1B gene expression, including Interferon Regulatory Factor 5 and Complement 3a Receptor 1. The preliminary results from our methods suggest that graphical models based on integrative genomic data are computationally efficient, work well with small sample size, and can describe complex interactions among genes and polymorphisms that could not be identified by pair-wise association tests. Supported by: P01HL083069 and R01HL086601.

Inference of human demographic parameters using haplotype patterns from genome-wide SNP data. *K. E. Lohmueller[1,2], A. Auton[2], C. D. Bustamante[2], A. G. Clark[1]* 1) Dept Mol Biol and Genetics, Cornell Univ, Ithaca, NY; 2) Dept Biol Stats and Comp Biol, Cornell Univ, Ithaca, NY.

Accurate inference of human demographic history from genetic data is essential for identification of single nucleotide polymorphism (SNP) association with disease and for inference of natural selection. Haplotype diversity and haplotype sharing carry additional demographic information to that obtainable from SNP frequency spectra, and so we propose a novel method using haplotype summary statistics to fit demographic models to genome-wide SNP data. We divide the genome into 0.25 cM windows and for each we tabulate the number of distinct haplotypes and the frequency of the most common haplotype. We summarize the data by the genome-wide joint distribution of these two statistics. Coalescent simulations are then used to evaluate whether different demographic models are compatible with the observed data. Application of our method to simulated data shows that our method can reliably infer parameters from complex demographic models (such as bottlenecks) and is relatively robust to the levels of SNP ascertainment bias found in many genome-wide datasets. We have applied our method to data collected by the International HapMap Consortium and find that a bottleneck model best fits the CEU population. We have also analyzed a large dataset consisting of Affymetrix 500k data from ~2,900 individuals with ancestry from Taiwan, Japan, India, Mexico and many European countries. Since this dataset includes ~2,300 European individuals, we are able to study haplotype patterns at a fine scale within Europe. Interestingly, we find that within Europe there is a south-to-north gradient with decreasing levels of haplotype diversity moving north, consistent with south to north migrations. We also find that the southwestern European sample has higher haplotype diversity than the southeastern European sample. Additionally, a higher proportion of haplotypes are shared between the southwestern European sample and the Yoruba sample than between southeastern European sample and the Yoruba sample. These two patterns are consistent with recent admixture across the Mediterranean from Northern Africa.

Managing incidental results of newborn screening: Health care provider reasoning about disclosure. *J. Allanson[1], F. Miller[2], R. Hayeems[2], J. Bytautas[2], J. Carroll[3], P. Chakraborty[1], R. Christensen[2], M. Paynter[2], J. Little[4], B. Wilson[4]*
1) Dept Genetics, Children's Hospital Eastern Ontario, Ottawa, ON; 2) Dept Health Policy, Management & Evaluation, University of Toronto, ON; 3) Dept Family & Community Medicine, Mount Sinai Hospital, Toronto, ON; 4) Dept Epidemiology & Community Medicine, University of Ottawa, ON.

Background: Attention to incidental results generated through newborn screening (NBS) is essential in an era of expanded technologic capacity (Bailey, 2008). The views of stakeholders are relevant to decisions about managing incidental results (e.g., carrier), but how attitudes should inform policy remains unclear. **Methods:** We conducted a mixed methods study of health care providers (HCPs) in Ontario, Canada with a potential role in NBS for sickle cell disorders (SCD), including: a cross-sectional survey of a stratified random sample of 7 HCPs (N=3,113), and open-ended, semi-structured interviews (N=47) with a purposeful sample of group members. In this setting, SCD was a new addition to the NBS panel, and the program did not routinely disclose carrier results. **Results:**With a response rate of 63%, support for reasons favoring disclosure was high (65-93%), as was disagreement with reasons opposing disclosure (67-90%). Genetics professionals expressed less support for arguments favoring disclosure (35-79%), and less disagreement with arguments opposing disclosure (29-75%). A slim majority of genetics professionals (52%) agreed that a reason to avoid disclosure was the importance of allowing the child to decide. Qualitatively, the dominant theme was a duty to disclose. For most HPCs, this duty was incumbent on the clinician: if possessed, carrier results could not be withheld. **Discussion:** While majority/ dominant opinion was clear, questions remain about how opinion should guide public policy, specifically: (1) how policy should balance descriptive ethics (i.e., what HCPs believe) with normative ethics (i.e., what duty-based principles require); (2) how dissenting opinion should be considered; and (3) how moral principles grounded in clinical obligations should inform policy for *public health* initiatives.

Chitinase-3-like 1 (CHI3L1) and schizophrenia: investigation of functional mechanisms for genetic susceptibility.

E. Kenny[1], M. Hill[1], S. Roche[2], M. Gill[1], D. W. Morris[1], R. Anney[1], A. Corvin[1], The International Schizophrenia Consortium 1) Neuropsychiatric Genetics Research Group, Dept. of Psychiatry and Institute of Molecular Medicine, Trinity College Dublin, Ireland; 2) Smurfit Institute of Genetics, Trinity College Dublin, Ireland.

Introduction: Genetic and gene expression data supports Chitinase-3-like 1 (CHI3L1), as a SZ candidate gene. Two studies report association with the same allele at a SNP (rs10399805) which disrupts the C/EBP-AML-1 binding site in the gene promotor and is predicted to increase CHI3L1 expression (Yang et al, 2008; Zhao et al, 2007). We investigated genetic association in the International Schizophrenia Consortium (ISC) sample and explored cis-acting regulation of CHI3L1 expression in lymphoblasts and post-mortem brain. Methods: We examined 13 SNPs at the locus using GWAS data from the ISC, a European consortium including >3,000 cases. We investigated allele specific expression imbalance (AEI) by measuring relative mRNA levels attributable to each SNP allele in RNA isolated from individuals heterozygous for a marker SNP within mRNA from lymphoblasts from the Caucasian HapMap sample (n=45) and post-mortem human brain tissue (BA46) (n=42). Results: We found no significant evidence for association at CHI3L1. We identified potent cis-acting variation regulating CHI3L1 expression in lymhoblasts, but not brain: this was mostly, but not exclusively, attributable to rs4950928. Conclusions: We did not confirm association with common variation at CHI3L1 in the ISC sample. SNP rs4950928 explains the majority of cis-acting variation at the locus in lymphoblasts; additional AEI observed is not attributable to common SNPs within +/- 10kb of the locus. CHI3L1 is subject to tissue/cell specific regulation and does not contain cis-acting variation influencing steady state transcription in post-mortem brain.

Association studies between clinical features of the Marfan syndrome spectrum within a cohort of 1013 patients with or another type I fibrillinopathy: a clue for a better understanding of the pathogenesis? *L. Faivre[1], G. Collod-Beroud[2], A. Child[3], A. De Paepe[4], C. Binquet[5], E. Gautier[5], C. Stheneur[7], C. Bonithon[5], M. Claustres[2], C. Beroud[2], L. Ades[6], C. Boileau[7], G. Jondeau[8]* 1) Dept Genetics, Dijon, France; 2) U827, Montpellier, France; 3) St. Georges, London, UK; 4) Ghent University Hospital, Belgium; 5) CIC-EC, CHU Dijon, France; 6) Sydney, Australia; 7) Génétique moléculaire, Hôpital Ambroise Paré, France; 8) Centre de Référence Marfan, Hôpital Bichat, France.

The cardinal features of MFS involve the ocular, cardiovascular and skeletal systems, as well as the skin, the lung and dural systems. Taking advantage of the data of a large collaborative study designed for a genotype-phenotype correlation study including 1013 probands with a pathogenic FBN1 mutation, we searched for an association between clinical features of the MFS spectrum. We first perform a multiple correspondence analysis in order to guide the choice of the associations to further test using chi-square tests. We found preferential associations within systems rather than between systems. Within the skeletal system, when considering a clinical feature typical of bone overgrowth in MFS such as arachnodactyly, we found a significant association between a majority of other skeletal features part of the Ghent criteria, including dolichostenomelia, pectus deformity, scoliosis, protrusio acetabule, joint hyperlaxity and facial dysmorphism ($p<0.001$). Not surprisingly, we found a significant association between ascending aortic dilatation (AAD) and aortic valve regurgitation ($p<0.001$) as well as between mitral valve prolapse and mitral regurgitation ($p<0.001$) or between striae and herniae ($p<0.001$). When studying associations between systems, we found a significant association between major skeletal involvement and AAD, striae, dural ectasia and pneumothorax ($p<0.001$). The search for other associations was non significant: in particular no association was found between ectopia lentis and other systems of the MFS spectrum. These results were concordant with the implication of the TGF in the cardiac, skeletal, dural and skin phenotype but not in the ocular phenotype.

Diversity profile of the human skin microbiome in health and disease. *E. A. Grice[1], H. H. Kong[2], S. P. Conlan[1], A. C. Young[3], N. I. S. C. Comparative Sequencing Program[3], G. G. Bouffard[3,4], R. W. Blakesley[3,4], M. L. Turner[2], J. A. Segre[1]* 1) Genetics and Molecular Biology Branch, NHGRI, NIH, Bethesda, MD; 2) Dermatology Branch, Center for Cancer Research, NCI, NIH, Bethesda, MD; 3) NIH Intramural Sequencing Center, NHGRI, NIH, Bethesda, MD; 4) Genome Technology Branch, NHGRI, NIH, Bethesda, MD.

The concept that the human body is host to trillions of microbes is revolutionizing our view of the human genome while underscoring the role of the gene-environment interface in complex disorders. One such disorder, with a known genetic component, is the very common inflammatory skin disorder atopic dermatitis (AD; eczema) whose incidence has tripled in the past 30 years. The skin is not only the first line of defense against invasion, but also host to a diversity of microbes associated with human health and disease. To assess cutaneous bacterial diversity and abundance, we employ 16S rRNA gene phylotyping. Our previous analysis of the most commonly affected human site in AD, the antecubital fossae (inner elbow), demonstrated a unique skin core microbiome dominated by *Janthinobacteria* and *Pseudomonas* (both phylum *Proteobacteria*) with less representation from five other bacterial phyla. Skin provides an unprecedented opportunity to sample multiple sites from the same individual, many with underlying left-right symmetry. Skin sub-sites have unique environmental niches: moist/dry, haired/non-haired, acid/basic, sebaceous (oily)/non-sebaceous. Associated with these specific areas are stereotyped human disorders; e.g. psoriasis affects outer elbow and eczema affects the inner elbow. We are currently ascertaining a wide physiological range of skin sub-sites from healthy humans to comprehensively survey the resident microbiota and address the fundamental question of whether there is a baseline cutaneous microbiome. This data is a foundation for our studies investigating alterations of skin microbiota in a disease state, specifically AD. Our long term goal is to elucidate the contribution of the cutaneous microbiome to complex skin disorders and translate this into novel pharmacological treatments.

Gene-gene interaction between 5-HT2A receptor (HTR2A) and HLA-DRB1 in rheumatoid arthritis. *L. Padyukov[1], H. Källberg[2], L. Alfredsson[2], L. Klareskog[1], M. Seddighzadeh[1]* 1) Rheumatology Unit, Karolinska Inst, Stockholm, Sweden; 2) Department of Environmental Medicine, Karolinska Inst, Stockholm, Sweden.

Background: 5-HTR2A is a gene of importance in schizophrenia but it has also been shown to be involved in the immunity. Previously we demonstrated that variations in the HTR2A gene are associated with rheumatoid arthritis (RA). However, since the association is moderate and RA is a complex disease we aimed to study the interaction between the HTR2A gene and HLA-DRB1-shared epitope (SE) alleles, a major genetic susceptibility risk factor for RA.

Methods: In the current investigation more than 3000 individuals from a Swedish EIRA study were included. Haplotype analysis was performed by HaploView and the individual haplotype was estimated using the PHASE. The degree of interaction was quantified by calculating the attributable proportion due to interaction (AP) together with 95% CI.

Results: The single nucleotide polymorphism (SNP) rs1328674 was shown to interact synergistically with HLA-DRB1 SE alleles with an AP = 0.6 (95% CI 0.4-0.9). Haplotype frequency analysis based on the SNPs rs1328674 and rs6314 showed lower representation of the TC haplotype in patients compared to controls (Chi-square=7.88, p=0.043). Moreover, this haplotype was interacting with HLA-DRB1-SE alleles in an antagonistic manner resulting in a decrease of the risk of developing RA from OR 2.8 to 1.8 (AP = 0.4 (95% CI 0.1-0.6).

Conclusions: The presents study demonstrates that there is a significant interaction between HTR2A gene and HLA-DRB1-SE alleles, which might have an impact on the risk of developing RA similar to the interaction between HLA-DRB1-SE alleles and PTPN22. The biological mechanisms of this interaction on the functional level remain to be studied.

MPO genetic variation is associated with birthweight, particularly among African American women. *G. K. Swamy[1], N. Ellis[2], M. E. Garrett[2], P. Maxson[3], M. L. Miranda[3], R. B. Williams[4], A. E. Ashley-Koch[2]* 1) Ob-Gyn, Duke University, Durham, NC; 2) Center for Human Genetics, Duke University Medical Center, Durham, NC; 3) Nicholas School of the Environment, Duke University, Durham, NC; 4) Behavioral Medicine Research Center, Duke University Medical Center, Durham, NC.

Oxidative stress is caused by an imbalance between the production of reactive oxygen and the ability to detoxify the reactive intermediates or repair the resulting damage. Elevated markers of oxidative stress have been associated with adverse pregnancy outcomes such as gestational diabetes and preeclampsia. We sought to determine whether genetic variability in oxidative-stress-related enzymes contributes to preterm birth (PTB 37 weeks gestation) and infant birthweight (BWT). The Healthy Pregnancy, Healthy Baby Study is a prospective cohort of pregnant women in Durham, NC aimed at identifying genetic, social and environmental contributors to disparities in pregnancy outcomes among women in the US South. Data and genotyping results were available for 621 women, of which 67% were non-Hispanic Black (NHB) race. Haplotype tagging SNPs were genotyped for 4 enzymes involved in the oxidative stress pathway - CYP2E1, CYP2A6, NQO1 and MPO. Using generalized estimating equations and controlling for race, maternal genotypes for each SNP were evaluated for association with PTB and BWT. Within the CYP2E1 gene, two SNPs were marginally associated with PTB (rs743535 p=0.03) and BWT (p=0.02). Within the MPO gene, SNP rs35921530 was strongly associated with BWT (p=0.006), with the association being primarily restricted to the NHB mothers (p=0.02). In addition to the oxidative stress pathway, MPO is involved in the inflammatory response as it is released from neutrophilic granules and exhibits significant antimicrobial oxidative activity. This is an intriguing finding, given the known associations between inflammation and adverse pregnancy outcomes. Future analyses will examine the role of gene x environment interactions, specifically with maternal infections and pollutant exposures, both of which have been associated with adverse pregnancy outcomes and oxidative stress.

A novel autosomal dominant cardiac arrhythmia of hisian type and associated dilated cardiomyopathy as a cause of sudden death. *S. Saal[1], L. Faivre[1], G. Bertaux[2], S. Falcon-Eicher[2], C. Thauvin[1], A. Masurel[1], P. Charron[4], P. Richard[4], C. De Chillou[3], S. Beziau[3], F. Kyndt[3], JJ. Schott[3], JE. Wolf[2], G. Laurent[2]* 1) Genetique, Dijon, France; 2) Cardiologie, Dijon, France; 3) Cardiologie, Nantes, France; 4) Cardiologie, La Pitie Salpetriere, France.

We report a novel autosomal dominant cardiac arrhythmia (CA) resulting in polymorphic ventricular ectopic beats (VEB) and non sustained ventricular tachycardia (NSVT) originated from the His bundle. In contrast to baseline rest, VEB and NSVT had the tendency to disappear during exercise. Familial investigations revealed 4 males and 4 affected females in three generations with an age at diagnosis ranging from birth to 37 years. The main symptomatology included palpitations, heart failure, but also syncopes in 2 patients and sudden death in 1 male at age 29 during rest. A dilated cardiomyopathy (DCM) was diagnosed in 3 females and was considered as a complication of CA since it never pre-existed to CA. Several antiarrhythmic drugs (AAD) were tried out in 2 females with permanent DCM but were poorly effective. Because of the bradycardia induced by AAD, a double chamber pacemaker was implanted. An additional cryoablation of the His bundle has also been planned for one patient because of unsatisfactory results. Electrophysiological investigations did not show any atrio-ventricular conduction abnormality. Neither rapid ventricular burst pacing, nor Isoproterenol infusion were able to induce more CA. Normalization of the surface ECG during exercise testing may be due to the sinus node overtaking control of the rhythm when reaching a certain threshold rate. Direct sequencing of the *Lamin A/C* and *ABCC9* genes was performed since these genes have been associated with CA and DCM, but was normal. The ankyrin-B gene sequencing revealed a previously described E1813K variant in the most severely affected patient but which did not segregate with the disorder. This variant could act as a modifier gene in this patient, but the major gene remained to be determined. The report of other families with such symptomatology would be helpful in order to better describe this new entity.

Familial autosomal dominant hyperinsulinism due to SUR1 (ABCC8) mutation. *A. G. Le Moing[1], G. Morin[1], C. Bellanné-Chantelot[2], F. Moreau[3], B. Demeer[1], M. Mathieu[1], A. Leke[3]* 1) Clinical Genetic Unit, University Hospital, Amiens, France; 2) Genetic Unit of Metabolic Disorders - Groupe Hospitalier Pitié-Salpétrière - Paris - France; 3) Neonatology - Department of Pediatrics - Amiens - France.

Familial congenital hyperinsulinism is responsible of prolonged hypoglycemia, secondary to mutations of genes involved in the regulation pathway of insulin secretion by Langerhans cells. Most of the cases are in relation with recessive mutations of two genes, located in 11p15.1, named SUR1 (ABCC8) and KIR6.2 (KCNJ11), and coding for two subunits of the same potassium channel. Dominant mutations of SUR1 (ABCC8) were only published in two families reported by Thornton (1998 and 2003) and Huopio (2000 and 2003). In these families, the hypoglycaemia was sensible to diazoxide. In the family reported by Huopio, the eventuality of transformation in diabetes with age was noticed. We report the observation of a female newborn presenting with severe hypoglycaemia at 36 hours of life, requiring continuous gastric nutrition at the beginning, but compatible with a normal breath feeding when diazoxide was started. Her father had similar symptoms in childhood, improved by diazoxide. In the two patients, a missense heterozygous mutation of SUR1 (ABCC8) was discovered (2143G>A - V715M). Screening of other genes involved in familial dominant hyperinsulinism was negative (HNF4, KIR6.2). The mutation was not present in the two paternal grand-parents of the index case.

A Posterior Probability of Linkage & Association Study of 111 Autism Candidate Genes. *F. Chen[2], N. Gharani[2], C. Dong[1], Y. Wang[1], D. Gordon[2], Y. Huang[4], J. Millonig[3], V. Vieland[4], H. Wang[1], J. Tischfield[2], T. Matise[2], L. Yu[2], W. Huang[1], L. Brzustowicz[2]* 1) Chinese National Human Genome Center, Shanghai, China; 2) Dept of Genetics, Rutgers University, Piscataway, NJ; 3) CABM, UMDNJ-RWJMS, Piscataway, NJ; 4) Research Institute at Nationwide Children's Hospital and Ohio State University, Columbus, OH.

Autism is a neurodevelopmental disorder with a complex genetic basis. We tested 111 biologically relevant candidate genes for genetic association with autism in 265 nuclear families from the Autism Genetic Resource Exchange (AGRE). Candidate genes were selected from three categories: 1) genes with similar function as the previously identified autism susceptibility gene ENGRAILED 2 (EN2); 2) genes from the serotonin and GABA neurotransmission pathways; and 3) genes from previously identified genomic linkage and association regions. Utilizing genotype data from the International HapMap Project, a total of 1497 tagSNPs were selected to efficiently capture the haplotype information of each gene. These SNPs were genotyped using the Illumina Bead Array assay and subsequently subjected to a strict QC and error checking procedure. Cleaned genotype data were analyzed by the Posterior Probability of Linkage (PPL) and the Posterior Probability of Linkage Disequilibrium (PPLD), which directly measure the probability of linkage and/or association. 8 SNPs from three gene regions on Chr 4, 6 and 7 showed 2-point PPL >10%. However, only the EPHB6-EPHA1 locus on Chr7 gave a multipoint PPL exceeding 5% (PPL=6.5%). Six additional SNPs from EPHB6-EPHA1 (Chr7) and MECP2 (ChrX) provided some evidence for association (PPLD=10%). Next we analyzed the recently released AGRE Affymetrix 500K SNP array data for our top candidate gene regions in our families. Evidence for association was obtained for 6 further SNPs at the 7q33-q35 region (PPLD range 21%~40%). Furthermore, some support for association (PPLD=9%) was detected at additional SNPs within MECP2. These preliminary data identify members of the Ephrin receptor tyrosine kinase gene family as potential novel candidate autism susceptibility loci and provide further support for a role of MECP2 in autism etiology.

Polymorphism in -synuclein gene (SNCA) confers susceptibility to late-onset Parkinson disease. *G. Wang, G. Mayhew, D. Casadesus, S. Zuchner, W. K. Scott, E. R. Martin, J. M. Vance* Inst Human Genomics, Univ Miami, Miami, FL.

The alpha-synuclein gene (SNCA) was originally found to cause the rare familial form of Parkinson disease (PD) by missense mutations and locus multiplication. Further studies suggest that common polymorphisms in SNCA, especially Rep1 in the promoter region, are associated with sporadic PD, though the association has not been consistent across different populations. To determine the association of SNCA variants with the common complex form of PD, we genotyped eight single nucleotide polymorphisms (SNP) located in the 3UTR and promoter regions in a family based sample set (727 families [307 singleton and 420 multiplex] , all are American whites). In the overall data set, we found strong associations between PD and four SNPs, including rs356165 ($p=1.1X10^{-5}$) in the 3UTR and 3 other SNPs (RS2619363, p=0.0094; RS2619364, p=0.013; RS2583988, p=0.023) in the promoter region. Interestingly, these associations vary after the sample set is stratified by age-at-onset. Specifically, the association is not significant in the early-onset (40 years old) subset, whereas the associations strengthen in the late-onset (>40 years old) subset. We suggest that these associated SNPs may confer susceptibility to the common complex late-onset form of PD by changing SNCA transcription levels through regulation of promoter activity or translation levels through modified miRNA targeting of the 3 UTR.

Genetic associations between dental caries in children and taste preference and saliva pathway genes. *S. Wendell[1], XJ. Wang[1], G. Barkanic[1], RJ. Weyant[1], R. Crout[2], D. McNeil[2], ML. Marazita[1]* 1) School of Dental Medicine, University of Pittsburgh; 2) School of Dentistry, West Virginia University.

Background: Dental caries (tooth decay) is one of the most common oral diseases and is influenced by a complex interplay of genetic and environmental factors. We investigated the association of caries with genes from multiple relevant oral processes including saliva, taste preference, and tooth structure. Methods: The Center for Oral Health Research in Appalachia (COHRA) recruits families in Northern Applachia, and collects biological samples, demographic data and clinical assessment of oral health. Genetic association was investigated using FBAT software TDT analysis of 29 SNPs in 24 genes. Phenotypic scores were no caries (score=0) and caries (score1). Analysis was conducted for two non-overlapping groups: children 6yrs (n=669 in 422 families); 7-15 (n=523 in 357 families), as well as the entire cohort All. Results: Three SNPs in taste receptor TAS2R38 were associated with protection from caries in 6yrs (p=0.006, 0.05, 0.04). A SNP in sweet receptor TAS1R2 was associated with protection in 6yrs (p=0.02) and borderline significant in 7-15yrs (p=0.06). GNAT3, a G-protein involved in downstream taste pathways, was associated with caries risk in 7-15yrs (p=0.04). The saliva pathways genes CALR and CACNA1A were associated with caries risk in 7-15yrs (both p=0.02). Additionally, NFATC4 was protective in 6yrs (p=0.04). Notably, the G allele of CACNB4 was also protective in 6yrs (p=0.04) while the C allele was borderline significant for risk in All (p=0.10). Proteinases MMP8 and CTSB were associated with caries risk in 7-15yrs (p=0.02, p=0.04) while MMP9 was protective in 6yrs (p=0.04). AMELX was associated with risk in 7-15yrs and All (p=0.03, p=0.04). Additional suggestive significance was seen in TUFT, AQP5, CACNA1A, NFATC4, MMP8, and CTSB. Conclusions: We have identified several significant associations of candidate genes from a variety of relevant oral processes with dental caries risk or protection. Notably, genes in the taste sensing pathways were associated with dental caries. NIH Grant # DE014899.

Replication stress induces submicroscopic copy number changes in normal human cells. *M. F. Arlt[1], J. G. Mulle[2], V. M. Schaibley[1], S. T. Warren[2], T. W. Glover[1]* 1) Dept. of Human Genetics, University of Michigan, Ann Arbor, MI; 2) Dept. of Human Genetics, Emory University, Atlanta, GA.

We previously reported that aphidicolin (APH)-induced replication stress leads to a high frequency of submicroscopic deletions, or copy number changes (CNCs), within the FRA3B fragile site that closely resemble those frequently found in cancer cells (Durkin et al. PNAS, 105: 246-251, 2007). We now show that replication stress leads to a high frequency of both deletions and duplications of tens to hundreds of kilobases across the entire human genome. These CNCs closely resemble normal copy number variants (CNVs) and *de novo*, pathogenic CNCs seen in humans with genetic and developmental disorders and in cancer cells.

We exposed normal human fibroblasts to APH-mediated replication stress and generated clonal cell populations. These clones were analyzed for CNCs using high-resolution array comparative genomic hybridization (aCGH). We identified and validated via a second technique 28 *de novo* CNCs (21 deletions and 7 duplications) from 8/14 (57%) clones isolated from the APH-treated fibroblasts and 2 *de novo* CNCs (2 deletions) from 1/11 (9%) clones from untreated controls. These CNCs ranged in size from 11 kilobases to over two megabases. To date, our efforts at sequencing deletion breakpoint junctions have yielded 6 breakpoint junction sequences. All of these breakpoint junctions are characterized by small (<5 bp) microhomologies, consistent with the hypothesis that these rearrangements are the result of NHEJ-mediated repair processes and not homologous recombination repair. Additional breakpoints are currently being sequenced. Our results demonstrate that replication stress induces a high frequency of submicroscopic CNCs in cultured normal cells that closely resemble those found *in vivo*. This is a previously unrecognized consequence of replication stress and our findings lead us to hypothesize that replication stress is an important mechanism in the formation of human CNVs and pathogenic CNCs.

Complete sex reversal in alpha thalassemia X-linked mental retardation (ATRX) syndrome. *K. Kessler[1], J.-S. Saldivar[2], L. Mehta[1]* 1) Div. of Medical Genetics, Schneider Children's Hospital, Manhasset NY; 2) Clinical Molecular Diagnostic Laboratory, City of Hope, Duarte, CA.

We describe an infant with a complex disorder of sexual development (DSD), presenting with complete sex reversal, developmental delays and dysmorphic features. This was the first pregnancy for a 28 y.o mother. Prenatal sonograms showed a left multicystic kidney and possible congenital heart defect. Amniocentesis revealed a 46,XY karyotype, though the fetus appeared female. FISH for SRY was positive and 7-dehydrocholesterol, 22q11 FISH and androgen receptor gene sequencing were normal. At birth, the infant had microcephaly and dysmorphic features, consisting of prominent occiput, short nose, downturned and open mouth, small jaw, low-set overfolded ears, and contracted digits. Genitalia appeared unambiguously female and a pelvic ultrasound did not show a uterus or any detectable gonads. Endocrine evaluation did not suggest any specific abnormalities. Cardiology evaluation noted a bicuspid aortic valve. On follow up the infant had significant developmental delays, failure to thrive, bilateral sensorineural deafness, high myopia and delayed visual maturation. Skeletal survey was normal. Chromosomal microarray analysis (Baylor version 6.2/100K array), and sterol profile were normal. ATRX gene sequencing detected a hemizygous deletion of exon 35, predicted to be deleterious. The exact boundaries of the deletion are unknown. RBC indices did not suggest alpha thalassemia, however an unexplained leukocytosis (30-50 K /uL) with high platelet count, was present. The parents went on to have another affected pregnancy which was terminated. ATRX is a syndromic DSD, but complete sex reversal, as seen in our patient, is only rarely reported. A severe genital phenotype is felt to correlate with mutations in the helicase domain of the ATRX gene (Gibbons et al, 2008), particularly in exons 35 and 36, and the findings in our case support this association. Discordance between fetal karyotype and sonographic gender, and genital abnormalities in a syndromic infant are important clues to early diagnosis of this severe mental retardation syndrome.

Patterns of association of microRNAs with schizophrenia symptoms. *M. Muinos-Gimeno[1,2], A. Brunet[1,3,4], J. Real[4], V. Vallès[5], A. Labad[6], R. Guillamat[5], M. Guitart[4], X. Estivill[1,2,3,7], Y. Espinosa-Parrilla[1,2]* 1) Dept Genes & Disease, Centre de Regulació Genòmica, Barcelona; 2) CIBERESP, CRG, Barcelona; 3) CeGen, CRG, Barcelona; 4) Corporació Sanitària Parc Taulí, Sabadell; 5) Consorci Sanitari de Terrassa, Terrassa; 6) Hospital Universitari Institut Pere Mata, Reus; 7) Pompeu Fabra University, Barcelona, Spain.

Schizophrenia (SZ) is a severe neuro-developmental disorder characterized by disturbances in nearly every function of the brain: cognition, perception, affect and thought. Albeit having a strong genetic disposition its aetiology is still largely unknown. MicroRNAs (miRNAs) are small non-coding RNAs that regulate the expression of many mRNAs. Little is known about the functional mechanisms regulated by miRNAs in the brain but miRNA mediated gene regulation has already been involved in the control of neuronal development and differentiation as well as synaptic plasticity. In addition, differential expression of several miRNAs in the pre-frontal cortex of SZ patients has been reported, suggesting that miRNAs might be good candidate genes for this disorder. In order to evaluate this hypothesis we constructed a panel of 768 SNPs covering miRNA regions (MiRBase 7.1) and genotyped a group of 341 controls and 235 patients with SZ (custom Golden Gate assay, Illumina). Association studies showed 8 miRNAs to be associated with SZ (p0.005, sex adjusted) and two other miRNAs to be strongly associated with age at onset, being the association significant after correction for multiple testing ($p<8.8x10^{-5}$). Moreover, we performed association analysis with different quantitative traits from the Positive and Negative Syndrome Scale (PANSS) and found a highly significant association for one miRNA with total PANSS score ($p=4.0x10^{-5}$). Furthermore, two other miRNAs were significantly associated ($p=1.0x10^{-5}$) with higher scores for the positive and disorganized factors extracted from factor analysis of PANSS. Interestingly, one of the latter miRNAs is differentially expressed in the prefrontal cortex of patients with SZ, further supporting a role for this miRNA in the aetiology of SZ. Our results propose miRNAs as important contributors to the genetic basis of SZ.

Human Subtelomeric Gaps and Structural Variation. *H. Riethman[1], E. E. Eichler[2], R. Wilson[3], T. Graves[3], C. Fronick[3], L. Courtney[3], S. Hu[1], S. Paul[1]* 1) Wistar Inst, Philadelphia, PA; 2) Dept of Genome Sciences and HHMI, University of Washington, Seattle, WA; 3) Washington University Genome Sequencing Center, St. Louis, MO.

Structural variation is abundant in subtelomeric DNA, and is likely to have important consequences for transcriptional activity as well as for regulation and stability of (TTAGGG)n tracts. Both copy number variation and alternative sequence organizations are major components of this structural variation. Our current focus is on DNA comprising the the most distal subtelomeric sequences, subterminal DNA. These regions are both more divergent than other subtelomeric repeat regions and very important for understanding telomere biology. Many (TTAGGG)n-adjacent sequences are still missing from the reference sequence itself, and a large number of structural variants carrying new common subterminal alleles remain to be discovered and characterized. We are using end-sequenced fosmid libraries from the Human Structural Variation Initiative to characterize subterminal structural variation. Importantly, because the libraries were prepared using sheared source DNA, terminal fosmids with one mate pair corresponding to part of the terminal (TTAGGG)n sequence tract can be identified. Several large structural variants and telomeric gap-spanning fosmids were identified and sequenced, extending the reference sequence to (TTAGGG)n tracts for these subterminal alleles. In addition, one fosmid library (G248) was exhaustively screened for terminal fosmids using its paired-end sequences; the global collection of subterminal alleles from this genome was characterized by restriction mapping and directed sequencing. Our results demonstrate the feasibility of isolating novel subterminal structural variants using this approach and show that, while distal terminal (TTAGGG)n tracts are truncated in the clones, subterminal DNA remains intact. We plan to systematically identify and characterize subterminal sequences from common telomere alleles, filling a major gap in our knowledge of subtelomere structures in the human genome and permitting development of new tools for studying telomere biology.

Linkage Analysis of a Large Tourette Syndrome Pedigree. *S. Knight[1], N. J. Camp[1], H. Coon[2], M. Johnsom[2], W. McMahon[2]* 1) Dept of Biomedical Informatics, University of Utah, Salt Lake City, UT; 2) Dept of Psychiatry, University of Utah, Salt Lake City, UT.

Tourette Syndrome (TS) is a neuropsychiatric disorder characterized by multiple motor or phonic tics that wax and wan over a lifetime. The heritability of TS has been well established, yet few genetic studies have found significant results or replicated results. The purpose of this study was to perform a genomewide linkage analysis to identify regions of the genome that may harbor susceptibility genes for TS. Our resource consisted of one large pedigree of 260 individuals that contains a total of 67 affected individuals with a definite TS diagnosis. Two-point and multipoint linkage analyses were performed on a 10cM spaced microsatellite autosomal marker set. Since the pedigree was too large to allow for analysis using exact methods, MCLINK, a Monte Carlo Markov chain method, was used for the multipoint analyses. General dominant and recessive affecteds only models were used, in addition to nonparametric linkage (NPL). Under a recessive model, there was significant linkage with a two-point LOD score of 3.44 on chromosome 3p; the multipoint maximum was 3.47 in this region. Under a dominant model, nominal linkage results were also found on 4q (multipoint LOD=1.57). The NPL results were a LOD equivalent of 1.12 for chromosome. 3 and 0.94 for chromosome 4. The linkage peaks on 3p and on 4q are in regions that have been previously reported in linkage studies of TS. The statistical recombinant mapping of chromosome 3 indicated the region of sharing was between 37 and 88 cM and 26 cases shared the same haplotype in this region.

Copy number variations in schizophrenia : from association to individual patterning. *F. Torri[1], S. Lupoli[1,2], A. Orro[3,5], S. Potkin[4], E. Salvi[1], P. Cozzi[5], A. Calabria[5], J. Turner[4], J. Fallon[4], B. Lerer[6], G. Guffanti[1], C. Barlassina[1], C. Cosentino[1], V. Tieran[1], F. Taddeo[1], L. Luciano[3], D. Cusi[7], F. Macciardi[1]* 1) University of Milan, Milan, Italy; 2) INSPE, Scientific Institute San Raffaele, Milan, Italy; 3) CILEA Consortium, Segrate, Milan, Italy; 4) University of California, Irvine,Usa; 5) ITB, CNR, Milan, Italy; 6) Hadassah-Hebrew University Medical Center, Jerusalem, Israel; 7) San Carlo Borromeo Hospital, Milan, Italy.

Copy Number Variations (CNVs) role in determining susceptibility to complex disorders is becoming a key hypothesis. The outstanding challenge is now to understand disease model underlying CNVs involvement in diseases like schizophrenia (SCZ). Beside the common variants - common disease hypothesis for complex diseases, the rare variants - common disease one is emerging centered on the possible interplay of a network of individual rare CNVs. Starting from the cytogenetic evidence of a deletion of ~3.5 Mb on chromosome 13q encompassing many genes in a patient with a bipolar-schizoaffective picture, we assessed CNVs patterns in an Illumina HumanCNV370 case control study of SCZ of ~200 individuals. We cross-validated our findings on a subset of patients using MLPA. To detect CNVs we used different analytical algorithms, the Hidden Markov Model based QuantiSNP and the Circular Binary Segmental modified implemented in Nexus (Biodiscovery Inc.). We found that schizophrenics tend to have significantly more deletions than controls (p=1E-21), encompassing both heterozygous and homozygous events. In particular our logistic regression approach showed that cases and controls differ mainly in terms of heterozygous deletion (p=1E-3). Moreover we detected a significant association to SCZ of CNVs mapping in genes involved in synapse functionality and axon guidance, even after correction for multiple testing. We also found that patients show other CNV regions, including a highly significant CNV on chromosome 8. Considering each single subject individual CNVs patterning, we found that several pathways involved in regulation of neurodevelopmental functions are significantly enriched in many patients.

Mutations in the *GGCX* and *ABCC6* Genes in a Family with Pseudoxanthoma Elasticum-like Phenotypes. *Q. Li[1], D. K. Grange[2], N. L. Armstrong[2], A. J. Whelan[2], M. Y. Hurley[3], M. Rishavy[4], K. Hallgren[4], K. L. Berkner[4], L. J. Schurgers[5], Q. Jiang[1], J. Uitto[1]* 1) Department of Dermatology and Cutaneous Biology, and Biochemistry and Molecular Biology, Jefferson Medical College, Philadelphia, PA; 2) Division of Gentics and Genomic Medicine, Department of Pediatrics, Washington University School of Medicine, St. Louis, MO; 3) Divisionof Dermatopathology, Department of Dermatology, St. Louis University School of Medicine, St. Louis, MO; 4) Department of Molecular Cardiology, Lerner Research Institute, Cleveland Clinic Lerner Colleg eof Medicine at Case Western Reserve University, Cleveland, OH; 5) Cardiovascular Research Institute and VitaK BV, University of Maastricht, Maastricht, The Netherlands.

A characteristic feature of classic PXE, an autosomal recessive disorder caused by mutations in the *ABCC6* gene, is aberrant mineralization of connective tissues. Here, we report a family with PXE-like cutaneous features in association with multiple coagulation factor deficiency, an autosomal recessive disorder associated with *GGCX* mutations. The proband and her sister, both with severe skin findings with extensive mineralization, were compound heterozygotes for missense mutations in the *GGCX* gene, which were shown to result in reduced -glutamyl carboxylase activity and in under-carboxylation of matrix gla protein. The probands mother and aunt, also manifesting with PXE-like skin changes, were heterozygous carriers of a missense mutation (p.V255M) in *GGCX* and a null mutation (p.R1141X) in the *ABCC6* gene, suggesting digenic nature of their skin findings. Thus, reduced -glutamyl carboxylase activity in individuals either compound heterozygous for a missense mutation in *GGCX* or with haploinsufficiency in *GGCX* in combination with heterozygosity for *ABCC6* gene expression results in aberrant mineralization of skin leading to PXE-like phenotype. These findings expand the molecular basis of PXE-like phenotypes, and suggest a role for multiple genetic factors in pathologic tissue mineralization in general.

A genome-wide association study of 2,300 migraine with aura patients and 9,300 population-based controls. *V. Anttila[1,2], U. Todt[3], T. Freilinger[4], M. A. Kaunisto[2,5], M. Kallela[6] for the International Migraine Genetics Consortium*
1) Wellcome Trust Sanger Institute, Cambridge, Cambridgeshire, United Kingdom; 2) Center of Excellence for Complex Disease Genetics, University of Helsinki, Helsinki, Finland; 3) Institute of Human Genetics, University Hospital of Cologne, Cologne, Germany; 4) Department of Neurology, Klinikum Grohadern, Ludwig-Maximilians-University Munich, Munich, Germany; 5) Folkhälsan Research Center, Helsinki, Finland; 6) Helsinki University Central Hospital, Helsinki, Finland.

Migraine affects 15% of the adult population and is the most common cause of chronic episodic severe headache. Despite a well-established genetic component based on family and twin studies, no variants influencing migraine susceptibility have been convincingly identified and no genome-wide association studies reported. Given its recent successes, we use a genome-wide association strategy to identify genomic variation predisposing to common forms of migraine. To obtain sufficient sample size for the study, we formed the International Migraine Genetics Consortium with participants from seven leading migraine research centers. The discovery sample consists of 1,000 Finnish and 1,300 German comprehensively phenotyped migraine with aura patients. Control genotypes consist of 6,000 Finnish and 3,300 Central European population-based individuals genotyped in other studies. Genotyping is performed using the Illumina platform, and association analyses are performed using the PLINK software. Best ranked signals from the discovery sample will be replicated in a follow-up sample of 7,000 migraine with aura cases from Leiden, the Netherlands, Brisbane, Australia, and the HUNT study in Norway, as well as 20,000 additional controls to assess the population impact of identified variants. Here, we present the most likely associated signals and estimates of their population-specific effects.

Construction of a weighted genetic score and integration with clinical risk factors to predict the presence of diabetes in the population. *V. E. Mooser[1], K. Song[1], N. Lim[1], X. Yuan[1], T. Johnson[2,3], A. Abderrahmani[2], P. Vollenweider[2], H. Stirnadel[1], S. Sundseth[1], P. Matthews[1], G. Waeber[2], L. R. Cardon[1], D. M. Waterworth[1], X. Lin[1]* 1) Genetics Division, Discovery Analytics, Worldwide Epidemiology and Clinical Imaging Center, GlaxoSmithKline, King of Prussia PA, RTP NC and London UK; 2) Division of Medical Genetics and Department of Medicine, CHUV University Hospital, Lausanne, Switzerland; 3) Swiss Institute of Bioinformatics Lausanne Switzerland.

Many susceptibility genes for Type 2 diabetes have been discovered recently. Individually, these genes only increase the disease risk minimally. The risk in subjects who carry risk alleles within multiple susceptibility genes, and the value added by genetic information over the clinical risk factors in the prediction of diabetes remain to be determined. We constructed an additive genetic score using the most replicated SNP within 15 diabetes susceptibility genes, weighting each SNP with its reported effect size , but using the conservative lower bound of the CI as the estimate. We first tested this score in a study nested within the population-based CoLaus study in Lausanne, Switzerland (n = 5479), comprising 325 diabetic individuals and 599 controls matched 1:2 for age, gender and BMI. The diabetes risk, after adjustment for family history of diabetes, was 2.9 (1.8-4.7, p = 0.000007) for individuals with a genetic score within the top quintile, compared to the bottom quintile. Adding the weighted genetic score to the clinical covariates improved the area under the ROC curve by 2.7% (0.8%-5.2%, p = 0.009, from 68.6% to 71.3%). A similar OR was observed when analyzing the entire CoLaus population; however the gain in ROC analysis was not significant. In this nested case-control study, a simple, weighted 15 SNP-based score provides additional information over conventional clinical predictors of prevalent diabetes. The cumulative effect of these SNPs is not mediated solely by an increase in BMI, but the score and BMI appear to have a synergistic effect on T2D risk.

Eliminating redundant SNPs improves power in Genome Wide Association analyses. *R. W. Davies, A. F. R. Stewart, L. Chen, R. Roberts, G. A. Wells* University of Ottawa Heart Institute, Ottawa, Ontario, Canada.

Background: Genome Wide Association analyses involve testing hundreds of thousands of SNPs; as such, meaningful interpretation of results requires correction for multiple testing. However, as many SNPs show very similar characteristics, applying corrections which assume independence of SNPs is overly harsh. Objective: To block together and remove redundant SNPs in a GWA dataset using population specific measures of LD from control genotype data. Methods: Written using R-2.5.1, for a given chromosome the program starts with the SNP with the lowest physical position. Scanning forward in a 1Mbp window, the first SNP whose r2 value with the original SNP exceeds a pre-specified threshold is blocked with the original; subsequent SNPs are added to the growing block if their r2 value with each block member exceeds the r2 threshold. Once the 1 Mbp window is exhausted, a representative SNP is chosen from the block, and the process continues with the next unselected SNP on the chromosome. Results: Using data common to Affymetrix 5.0 and 6.0 arrays from our ongoing GWA, the Ottawa Heart Genomics Study, yields 482,251 SNPs from 1542 cases and 1422 controls. Removing the X chromosome, correcting for 5% MAF cases and controls, p0.001 HWE in controls and call rate 95% left 324,492 SNPs. Using a stringent r2 threshold of 0.90 on the refined dataset leaves 207,340 SNPs, a 36% reduction, while a threshold of 0.80 leaves 184,130 SNPs, a 43% reduction. Using Hapmap genotype data on the same SNPs gives reductions of 37% and 43% for thresholds of 0.90 and 0.80, respectively. Preliminary analysis of 2.5M potential SNPs for use in imputation, run using Hapmap genotype data on chromosomes 10 and 11, suggests a reduction of about 62%. A relative increase of 56% in the Bonferroni corrected p-value is realized with the removal of 117,152 SNPs from our data with an r2 threshold of 0.90. Conclusions: With a negligible loss of information, redundant SNPs can be removed from a GWA dataset, allowing for a less stringent correction for multiple testing and more power for a given sample size.

Mutatome of microsatellite unstable colorectal cancer. *P. Alhopuro[1], H. Sammalkorpi[1], J. Saharinen[2], D. Phichith[3], HL. Sweeney[3], D. Arango[4], A. Karhu[1], LA. Aaltonen[1]* 1) Department of Medical Genetics, University of Helsinki, Finland; 2) Biomedicum Bioinformatics Unit, University of Helsinki, Finland; 3) Department of Physiology, University of Pennsylvania, USA; 4) CIBBIM, Vall d'Hebron Hospital Research Institute, Spain.

Defects in the mismatch repair (MMR) system lead to microsatellite instability (MSI). MSI underlies the malignant progression of hereditary non-polyposis colorectal cancer and a subset of sporadic tumors, including colorectal cancer (CRC). Short repetitive DNA sequences in coding regions are prone to strand slippages under MMR deficiency and therefore frameshift mutations commonly occur in MSI tumors. In the MSI pathway a large number of random mutations are generated throughout the genome. Mutations that result in malignant advantages are selected for and show as high mutation frequencies in MSI cancers. The aim of this work was to identify novel MSI tumor suppressor target genes, and to gain understanding of the mutatome of MSI CRC. We analyzed mutation frequencies in coding microsatellites of 709 genes chosen based on expression array data (reduced expression in MSI CRC) and existence of a mononucleotide repeat. The mutation analysis was performed in 30 MSI CRCs and the genes that showed a somatic mutation rate 20% were evaluated in an extended set of 70 MSI CRCs. Normal tissue DNA was analyzed to confirm the somatic origin of mutations. The genes showing a somatic mutation rate 20%, were sequenced for somatic mutations in the entire coding region in 30 microsatellite stable (MSS) CRCs. We identified 16 novel candidate MSI target genes, which were somatically mutated in 23-55% of MSI CRCs. One of the genes identified was MYH11 encoding smooth muscle myosin, which was somatically mutated in 55% of MSI CRCs. MYH11 mutations were also identified in MSS CRC, as well as in the germline of a patient with Peutz-Jeghers syndrome. Functional analysis of MYH11 mutations suggests a role for unregulated myosin in CRC development. This is the first unbiased work to characterize somatic mutation rates in MSI cancer giving significant insight into MMR driven tumorigenesis and increasing our knowledge on mutation rates in cancer.

Differentiated SNP Selection and Multivariate Visualization of Structured Populations in Association Studies. *K. Miclaus[1,2], R. Wolfinger[2]* 1) Statistics Dept, North Carolina State University, Raleigh, NC; 2) JMP Genomics, SAS Institute Cary, NC.

In the new era of large-scale, collaborative Genome Wide Association Studies (GWAS), population stratification has become a critical issue that must be addressed. In order to build upon the methods developed to control the confounding effect of a structured population, it is extremely important to visualize and quantify that effect. In this work, we develop methodology for SNP selection and subsequent visualization based on deviation from Hardy-Weinberg Equilibrium in conjunction with Non-metric Multidimensional Scaling; a distance-based multivariate technique. Current preferred methodology advises the use of independent markers to avoid confounding, yet this is computationally difficult in studies with many markers and individuals and researchers may resort to random SNP selection for simplicity. Through simulation, it is shown that SNP selection based on HWD is robust against confounding linkage disequilibrium patterns that have been problematic in past studies and methods. Results show that selection based on HWD yield a set of substructure informative SNPs that contain a highly significant smaller proportion of SNPs that are from confounding LD regions compared to random SNP selection. Additionally HWD selected SNPs results in a significantly higher measure of differentiation, F_{ST}, on average than randomly chosen SNPs as well as randomly chosen SNPs that were NOT in a confounding LD region. Non-metric MDS is shown to be an appropriate multivariate visualization tool in conjunction with HWD SNP selection through theoretical and empirical study from HapMap samples. Comparisons show that the standard method PCA may find differentiation in an unstructured population due to the nature of maximizing explained variance by the eigenvectors, while MDS finds coordinates that attempt to encompass the true structure of the data. Results give promise to the development of future methods to correct for population stratification in association testing based on these methodologies.

Multiple approaches to use GWA data to identify variants impacting on age of type 2 diabetes diagnosis. *M. McCarthy[1,2], I. Fernandez-Cadenas[2,3], I. Prokopenko[2], N. Timpson[2,4], V. Boraska[2,5], N. Rayner[2], A. Hattersley[6], T. Frayling[6], E. Zeggini[2], C. Lindgren[1,2]* 1) OCDEM, University of Oxford, UK; 2) WTCHG, University of Oxford, UK; 3) LIN, HVH, Spain; 4) MRC Center for Causal Analyses in Translational Epidemiology, Bristol University, UK; 5) University of Split, Department of Biology, Croatia; 6) Peninsula Medical School, Exeter, UK.

Many common variants influencing T2D have been identified through GWA studies. This study used data from the Wellcome Trust Case Control Consortium (1924 cases, 2938 controls; 500k Affymetrix chip) to identify variants influencing T2D age of diagnosis (AoD). We examined: (a) T2D associations after stratifying cases by median AoD (51 years, 1002 early-AoD; 922 late-AoD cases) compared to 2938 WTCCC controls; (b) AoD associations by comparing early- and late-AoD cases, and by continuous trait analyses of AoD within cases; (c) evidence for AoD-dependent heterogeneity in T2D effect size by Breslow-Day analysis of early-AoD/control and late-AoD/control comparisons. These analyses identified several interesting signals associated with AoD itself, or for which T2D association was influenced by case AoD stratum. Amongst the former were rs6068566 (near *TSHZ2*), which showed significant differences in genotype frequency between early- and late-AoD cases ($p=3.7x10^{-6}$) and with AoD as a continuous trait ($p=7.9x10^{-5}$); and rs7317049 (near *SPRY2*) associated with AoD on the continuous ($p=9.9x10^{-7}$) and dichotomous ($p=2.8x10^{-4}$) tests. The most promising signal for AoD-stratum related differences in T2D association lies at rs11155055 (near *PRDM13*: Breslow-Day $p=1.1x10^{-6}$, late-AoD cases/controls, $p=7.4x10^{-4}$, early-AoD cases/controls, p=0.85). Another signal after stratification lies at rs10806665 (near *THBS2*: $p=3.4x10^{-8}$ for T2D association in early-AoD cases only) but was not significant in the Breslow-Day test (p=0.06). None of the previous established genes for T2D were associated with AoD or showed heterogeneity of T2D effect size by AoD-stratum. Whilst replication in additional data sets will be required to substantiate these findings, the multifaceted approach has identified several candidate regions potentially involved in influencing AoD of T2D.

VDR genetic variation is associated with birthweight among African American mothers. *M. E. Garrett[1], G. K. Swamy[2], N. Ellis[1], P. Maxson[3], M. L. Miranda[3], R. B. Williams[4], A. E. Ashley-Koch[1]* 1) Center for Human Genetics, Duke University, Durham, NC; 2) Ob-Gyn, Duke University Medical Center, Durham, NC; 3) Nicholas School of the Environment, Duke University, Durham, NC; 4) Behavioral Medicine Research Center, Duke University Medical Center, Durham, NC.

The Vitamin D receptor (VDR) mediates Vitamin D activity and its primary role in calcium homeostasis. However, research suggests that polymorphisms in the VDR gene modulate circulating levels of lead, a heavy metal that has been associated with adverse pregnancy outcomes such as preterm birth and low birthweight. Furthermore, higher blood lead levels have been documented among Black women as compared to White women in the US. It is unclear if these higher lead levels are due to differential environmental exposure, to genetic variability, or a combination of both. Therefore, we hypothesize that genetic variation in the VDR gene may contribute to the racial disparity in preterm birth (PTB 37 weeks gestation) and infant birthweight (BWT). The Healthy Pregnancy, Healthy Baby Study is a prospective cohort of pregnant women aimed at identifying genetic, social and environmental contributors to disparities in pregnancy outcomes among women in the US South. Data and genotyping results were available for 621 women, of which 67% were non-Hispanic Black (NHB) race. Haplotype tagging SNPs were genotyped for VDR via Taqman assays. Using generalized estimating equations and controlling for race, the mothers genotype for each SNP was evaluated for association with PTB and BWT. Of the 19 SNPs examined, two SNPs were associated with PTB (rs2853559 p=0.03) and BWT (rs7975128 p=0.04), respectively. In the NHB subset, two SNPs were strongly associated with BWT only (rs7975232 p=0.009; rs7975128 p=0.003). Although the mechanisms by which VDR genetic variation may contribute to pregnancy outcomes is unknown, the strong association with BWT among NHB women may function through altered lead metabolism and blood lead levels. Future analyses will examine the association between VDR genetic variability and circulating maternal blood lead levels and gene x environment interactions, particularly heavy metals exposures.

Association between genetic polymorphisms of Protease Inhibitor 3 and risk of acute respiratory distress syndrome. *P. Tejera[1], Z. Wang[1], R. Zhai[1], L. Su[1], CC. Sheu[1], DC. Christiani[1,2]* 1) Harvard School of Public Health, Boston, MA; 2) Massachusetts General Hospital, Boston, MA.

Background: Proteinase inhibitor 3 plays important roles in infection and inflammation. Our previous genome-wide gene expression analysis has revealed that PI3 was down-regulated in patients with acute respiratory distress syndrome (ARDS). In this study we hypothesized that genetic variations in PI3 might be associated with ARDS risk. Methods: We resequenced the entire PI3 gene in 29 healthy Caucasians. Based on the results of resequencing, haplotype-tagging single nucleotide polymorphisms (SNPs) were selected for genotyping. An additional four SNPs located in the promoter and in the 3 region of PI3 were also included. The study population included 441 ARDS cases and 1023 critically ill patients, nested within a prospectively enrolled cohort of the Molecular Epidemiology of ARDS study (Massachusetts General Hospital, Boston, MA). The associations between PI3 variants and ARDS risk were estimated by multivariate logistic regression models. Results: We identified 24 polymorphisms, 21 SNPs and 3 in/del, in the PI3 gene. Among them, ten were novel variants not previously described in Caucasians. Three tagging-SNPs (rs1983649, rs6032040, rs2664581) and four additional SNPs (A-1077G, rs35632684, rs17333381 and rs2267864) were genotyped and analyzed for the association with ARDS. We found that the variant genotypes of PI3-4 (OR, 1.35; 95% CI, 1.08-1.69; p=0.009), rs2664581 (OR, 1.36; 95% CI, 1.10-1.69; p=0.005), rs17333381 (OR, 1.34; 95% CI, 1.08-1.67; p=0.009) and rs2267864 (OR, 1.31; 95% CI, 1.06-1.62; p=0.012) were significantly associated with increased ARDS risk in both crude and adjusted analyses. In subgroup analyses, we found the associations of PI3 variants with ARDS development were even stronger in subjects aged<65 years and in subjects with risk factors for indirect lung injury (e.g., sepsis). In haplotype analysis the haplotype GTTTCTG was associated strongly with a higher risk of ARDS in subjects aged<65 (OR, 1.63; 95% CI, 1.14-2.32; p=0.007). Conclusion: Polymorphisms in PI3 gene are significantly associated with ARDS risk, particularly in subjects aged < 65.

Influence of genetic polymorphisms in circadian rhythm-related genes on polysomnographic sleep parameters in patients with primary insomnia. *K. Mack, S. N. Mitkus, A. Thompson, N. Wang, G. Birznieks, C. Lavedan* Vanda Pharmaceuticals Inc., Rockville, MD.

Insomnia is a highly prevalent sleep disorder, estimated to affect 30% of the adult population worldwide. Recent advances in circadian rhythm biology have identified genetic variations in several key molecular clock genes that are associated with circadian rhythm disorders and/or that influence sleep-wake behavior in healthy individuals. However, it is not known whether these genes play a role in insomnia. We have analyzed the effect of several polymorphisms in clock genes, including the period genes *PER2* and *PER3*, the circadian locomoter output cycles kaput *(CLOCK)* gene, and the gene for the adenosine A2a receptor *(ADORA2A)* on sleep parameters in 192 individuals between the ages of 18 and 64 years diagnosed with primary insomnia. Polysomnographic assessments measuring sleep efficiency, onset and maintenance, and sleep architecture were conducted in a sleep laboratory for 2 consecutive nights. Diurnal preference using the Owl-Lark questionnaire was also evaluated. Polymorphisms in several clock genes were found to influence sleep characteristics as well as diurnal preference in patients with insomnia. These results indicate that polymorphisms in sleep-regulating genes may not only play a role in advanced/delayed sleep phase syndrome but may also be important in primary insomnia. These findings also suggest contributions of common polymorphisms to the pathophysiology of both circadian rhythm disorders and primary insomnia. A greater understanding of clock-related genes may provide insights into the etiology of sleep disorders.

Conditional Mutant Mouse Models of Human Disease. *S. Rockwood, R. Babiuk, J. Eppig, C. Heffner, C. Lutz, S. Murray, M. Ringwald, M. Sasner, Y. Sharma* Genetic Research Science, The Jackson Laboratory, Bar Harbor, ME.

Genetically modified mice have greatly contributed to our understanding of basic biology and disease processes. In order to facilitate access to these mice, the Mouse Repository at The Jackson Laboratory was established to serve as a centralized resource for the archiving and distribution of genetically engineered mice at a high health status. As the number and complexity of alleles increase, so does the importance of providing adequate information to facilitate their selection and use. Conditional mutants represent a significant portion of the new alleles reposited; many have floxed alleles and serve as models of human disease such as: *Smn1*, Spinal Muscular Atrophy; *Mecp2*, Rett Syndrome; *Sirt1*, aging research; the RosaHD strain (JAX Stock7708) that expresses a neuropathogenic polyQ-mutant variant of the human Huntingtin protein (mhtt-exon1; 103Q) upon exposure to Cre recombinase. Others are experimental tools such as Brainbow mice (JAX Stocks 7901,7910,7911,7921), where Cre-induced recombination causes individual neurons to be uniquely and stably labeled with one of over 100 colors to enable studies of neuronal lineage and connectivity. Equally important are the Cre-expressing strains that are used in conjunction with these mutants. Because most of the existing Cre strains have not been fully characterized and ectopic expression of Cre can confound analysis of experimental results, we have undertaken a project to comprehensively characterize Cre activity in embryonic and adult mouse tissues. We will present a database of Cre expression data that includes images and expression patterns curated with terms from the Mouse Anatomical Dictionary, and an online interface searchable by site of Cre expression in various developmental stages. Donating a strain to the Repository fulfills the NIHs requirements for sharing of mice. Researchers wishing to have strains considered for inclusion in the Repository may submit their strains at: http://www.jax.org/grc/index.html. Support for this project has been provided by the NCRR (RR09781, RR11083, RR16049), NIA, HHMI, The Ellison Medical Foundation and from private charitable foundations.

Joint analysis of germline variants in the IGF pathway and prostate cancer risk. *S. Lindstrom, P. Kraft* Harvard School of Public Health, Boston, MA.

Technological advances have enabled researchers to genotype large numbers of Single Nucleotide Polymorphisms (SNPs) for assessment of association with different outcomes. These variants are often tested individually but joint analysis of multiple SNPs may be more powerful to detect association if marginal effects are small and interaction effects exist (i.e. epistasis). However, the ability of detecting SNP-SNP interaction is highly dependent on several factors including allele frequency, penetrance, number of causal loci and sample size. The insulin-like growth factor (IGF) pathway has consistently been implicated in prostate cancer. Both plasma levels of IGF1 in blood and genetic variation in genes involved in IGF metabolism have been associated with disease. To elucidate the role of genetic variation involved in the IGF pathway in prostate cancer, we genotyped 591 SNPs from a total of 24 genes in the Breast and Prostate Cancer Cohort Consortium (BPC3). The BPC3 constitutes of seven prospectively collected nested case-control studies, including in total 6,600 prostate cancer cases and 7,500 controls of Caucasian origin. We performed a comprehensive investigation by applying four different statistical methods for simultaneously analyzing multiple genetic variants. These methods included single SNP analysis, forward stepwise logistic regression and kernel machine procedures. We performed the analysis gene by gene as well as on an overall pathway level. Overall, we found no association with the IGF pathway and prostate cancer risk. We discuss the obstacles faced when analyzing multiple SNP interactions on a large scale basis including the impact of missing data, data complexity and computational burden. We also emphasize the importance of large-scale datasets such as the BPC3 in order to identify higher-order interactions in genetic epidemiology. We gratefully acknowledge the BPC3 investigators for access to data.

Pharmacogenetic studies in patients with temporal lobe epilepsy. *M. S. Silva[1], K. M. Siqueira[1], E. Bilevicius[2], R. Secolin[1], F. Cendes[2], I. Lopes-Cendes[1]* 1) Departament of Medical Genetics, Faculty of Medical Sciences, University of Campinas - UNICAMP - Campinas, São Paulo, Brazil; 2) Department of Neurology; Faculty of Medical Sciences, University of Campinas - UNICAMP, Campinas, São Paulo, Brazil.

Rationale:Mesial temporal lobe epilepsy (MTLE), the most common form of partial epilepsy, is associated with a high proportion of drug-resistant patients. One of the theories to explain the lack of success in the clinical treatment of patients with MTLE suggests that drug transporter genes overexpressed in the blood-brain barrier, such as members of the ATP-binding cassette (ABC) family, could decrease the entry of antiepileptic drugs (AEDs) in the central nervous system (CNS). Furthermore, there is functional evidence that genetic variation, such as single nucleotide polymorphisms (SNPs), can affect the expression of drug transporter genes. The purpose of this study was to evaluate whether SNPs in two drug-transporter genes: ABCB1 and ABCC2, could be associated with pharmacoresistance to AEDs in a large group of patients with MTLE. Methods:We included a total of 101 drug-resistant and 64 drug-responsive MTLE patients. We genotyped 5 SNPs from the dbSNPs database in the ABCB1 gene, and 6 dbSNPs from ABCC2 gene. Genotyping was carried out using the TaqMan system (Applied Biosystems). The significance of allelic association was assessed using logistic regression, logistf function in R environment. Results:Sample power calculation showed an statistical power of 0,98 for the sample studied. Logistic regression showed no significant association between any of the SNPs studied in ABCB1 gene and pharmacoresistance to AEDs. However, logistic regression showed a significant association between an intronic SNP (rs3740067) at the ABCC2 gene and pharmacoresistance to AEDs (p=0.0015); OR = 4.90 (95% CI: 1.48 - 16.2). Conclusion:We could not confirm the previous reported association between polymorphisms in the ABCB1 gene and pharmacoresistance to AEDs in our cohort of patients with MTLE. However, we found a significant association with an intronic SNP in the ABCC2 gene and pharmacoresistance in this group of patients.

microRNAs deregulation in disease progression and neuronal dysfunction in Parkinsons disease. *E. Minones-Moyano[1], S. Porta[2,1], M. Banez-Coronel[1], X. Estivill[1,2,3], E. Marti[1]* 1) Genes and Disease program, Center for Genomic Regulation (CRG-UPF), Barcelona, Barcelona, Spain; 2) Public Health and Epidemiology Network Biomedical Research Center (CIBERESP); 3) Pompeu Fabra University (UPF).

MicroRNAs (miRNAs) are small non-coding RNAs that modulate gene expression. Recent studies link miRNA deregulation to psychiatric disorders and neurodegenerative diseases, including Parkinson disease (PD). The characteristic neuropathological changes associated to PD progress in a topological predictable manner. The degeneration of the nigrostriatal dopaminergic pathway leads to disabling motor manifestations that characterize the symptomatic phase of the disease. The expression levels of some of genes relevant to PD neuropathology, in particular PARK2, PARK7 and SNCA, have been associated to changes in the cell susceptibility to stressing stmuli, such as oxidative stress. In addition, deregulation of the transcriptome has been widely reported in PD brains. Whether these altered expression profiles are associated to miRNA deregulation or dysfunction has not yet been addressed. Here we have analyzed miRNA expression profiles in brain samples of PD to discover gene expression networks modulated by miRNAs relevant in PD neuropathology. Our results show strong downregulation of a specific miRNA in amygdala samples of PD patients at the neuropathological Braak stage V. In the same patients this miRNA was also significantly downregulated in the frontal cortex, an area that presents mild inclusion body pathology. The expression of this miRNA in the cerebellum, a non-affected area, was similar to the control samples. Functional studies showed that downregulation of this miRNA in differentiated neuroblastoma cells leads to a moderate decrease in cell viability. Together, these results suggest that the downregulation of this miRNA positively correlates with the neuropathological changes associated with PD. Changes in the expression of this miRNA may underlie the initial transcriptome deregulation in PD, leading to neuronal dysfunction and disease progression.

Association of MHC region SNPs with airflow obstruction in severe Alpha 1-Antitrypsin Deficiency. *D. DeMeo[1], E. J. Campbell[2], S. I. Rennard[3], T. M. Delorey[1], A. F. Barker[4], M. L. Brantly[5], E. Eden[6], N. G. McElvaney[7], R. A. Sandhaus[8], J. M. Stocks[9], J. K. Stoller[10], C. Strange[11], G. Turino[6], E. K. Silverman[1]* 1) Dept Medicine, Channing Laboratory, Boston, MA; 2) U of Utah; 3) U of Nebraska; 4) Oregon Health and Science U; 5) U of Florida; 6) St. Luke's/Roosevelt; 7) Beaumont Hospital, Dublin; 8) National Jewish Medical and Research Center; 9) U of Texas at Tyler; 10) Cleveland Clinic; 11) Medical U of South Carolina.

Severe alpha 1-antitrypsin deficiency (AATD) (OMIM 107400) is an autosomal recessive condition characterized by variable susceptibility for the development of chronic obstructive lung disease (COPD) in individuals homozygous (PI ZZ) for the deficiency allele. Recently, it has been suggested that COPD may be an autoimmune disease. Although many autoimmune diseases have demonstrated association with Major Histocompatibility Complex (MHC) loci, to date there have been no large scale studies of polymorphic variation in MHC loci in individuals with AATD. We hypothesized that polymorphic variation in the MHC gene rich region of chromosome 6 contributes to the variable susceptibility to develop COPD in PI ZZ individuals. In a cohort of 378 PI ZZ individuals from 167 families, we evaluated 2,360 MHC region SNPs using the Illumina MHC Combined Panel set. All analyses were performed using the pedigree-based association test (PBAT) with covariates for smoking. Spirometric phenotypes included pre- and post-bronchodilator forced expiratory volume in one second (FEV1) as a percent of predicted and the ratio of FEV1/Forced vital capacity (FVC), both key intermediate phenotypes of COPD. Significant associations with spirometric phenotypes ($p<.01$) were identified for SNPs in multiple HLA loci, including HLA-A,-B,-C,-E,-F,-G and -DRA. SNPs in HLA-C approached the threshold for significance defined by a Bonferroni correction for 2,360 SNPs ($p<10-5$). We conclude that HLA genes may be important modifier genes in severe AAT deficiency. These findings suggest that genetic features of autoimmunity may have relevance for the development of COPD. Funding: HL072918, HL68926.

Combined mRNA and miRNA expression profiling of the CNGA3 mouse - a mouse model of cone dystrophies.

M. Bonin[1], K. Schäferhoff[1], S. Poths[1], S. Michalakis[2], M. Biel[2], O. Riess[1] 1) Medical Genetics Dept, Inst. Human Genetics, Tuebingen, Germany; 2) Munich Center of Integrated Protein Science and Dept. of Pharmacy, Munich Center of Integrated Protein Science and Dept. of Pharmacy.

The CNGA3-/- mouse is an animal model lacking the A subunit of the cone specific cyclic nucleotide gated channel. The phenotype is characterized by a loss of cone photoreceptor function and a progressive degeneration of the cones. To elucidate the biological events leading to the loss of photoreceptors we combined mRNA expression experiments with whole genome miRNA expression profiling. Expression analysis of CNGA3-/- and wildtype retinas in 2 age stages was performed using Affymetrix MOE 430 2.0 microarrays. Differential regulated transcripts with a minimum change in expression level of 1.5 fold with a p-value less than 0.05 were obtained and gene regulation networks were generated by the Ingenuity Pathways Analysis software. To verify the data 20 transcripts per time point were analyzed by qRT-PCR. miRNA expression profiling was conducted on a whole genome mouse miRNA array. 496 transcripts were differentially regulated in the retinas of the 4 week old mice and 204 in those of the 8 week old animals. Gene regulation networks revealed misregulations of genes associated with RNA post-transcriptional modification and cellular growth. 90 % of the transcripts chosen for real-time validation could be verified. In the miRNA array analysis of 4 week old mice we found 27 differently regulated miRNAs which have potential target genes included in the differential gene list of our previous transcriptional analysis. Expression analysis of the CNGA3-/- mouse highlighted a misregulation of the phototransduction cascade in accordance with the loss of visual function that characterizes the phenotype. The combination of mRNA and miRNA expression profiling permits a closer monitoring of the neurodegenerative events in the retina occurring during the course of degeneration.

Mutations in the Valosin Containing Protein Gene Result in Vacuolization of Human Myoblasts and Deficiencies in Multiple Cellular Processes. *J. Vesa[1], H. Su[1], W. Fan[2,3], GD. Watts[4], S. Krause[5], MC. Walter[5], JH. Weiss[6], DC. Wallace[2,3,7], VE. Kimonis[1]* 1) Dept. of Pediatrics, Div. of Genetics and Metabolism, University of California, Irvine, CA; 2) Center for Molecular and Mitochondrial Medicine and Genetics, University of California, Irvine, CA; 3) Dept. of Biological Chemistry, University of California, Irvine, CA; 4) Dept. of Orthopedic Surgery, Children's Hospital Boston, Harvard Medical School, Boston, MA; 5) Dept. of Neurology, Ludwig-Maximillians-University, Munich, Germany; 6) Dept. of Anatomy and Neurobiology, University of California, Irvine, CA; 7) Depts of Ecology and Evolutionary Biology and Pediatrics, University of California, Irvine, CA.

Inclusion body myopathy associated with Pagets disease and frontotemporal dementia (IBMPFD) is caused by mutations in the valosin containing protein (VCP). A mutated gene results in progressive proximal muscle weakness, inclusions and vacuoles in muscle fibers, malfunction in the bone remodeling, and premature frontotemporal dementia. VCP is involved in several cellular processes including the degradation of defective proteins. We have studied cellular consequences of VCP mutations in human primary myoblasts. Our results reveal that patients myoblasts accumulate large ubiquitin positive vacuoles that are able to fuse with lysosomes. Lysosomal membrane proteins Lamp1 and Lamp2 are defectively N-glycosylated in patients myoblasts. The maturation processes of mutant cells are affected suggesting that the myopathy in IBMPFD patients may be caused by a defective myotube formation. Additionally, mutant myoblasts show decreased proliferation activity and increased autophagy when cultured in the absence of nutrients, as well as increased apoptosis. Mitochondrial enzyme activities are upregulated in mutant cells suggesting that they attempt to compensate the cumulative energy deficiency. Our results elucidate that VCP mutations result in disturbances in several cellular processes in IBMPFD myoblasts, and these myoblasts can be used to clarify pathological mechanisms resulting in muscle weakness in patients. The observed cellular phenotype of mutant myoblasts can also be utilized as a model to help develop new therapies for IBMPFD.

Mutational spectrum of *TCF4* and cardinal manifestations of Pitt-Hopkins syndrome. *I. Giurgea[1], C. Missirian[2], P. Cacciagli[2], S. Whalen[1], T. Fredriksen[1], T. Gaillon[1], J. Rankin[3], M. Mathieu-Dramard[4], G. Morin[4], D. Martin-Coignard[5], C. Dubourg[6], B. Chabrol[2], J. Arfi[2], F. Giuliano[7], J.-C. Lambert[7], N. Philip[2], P. Sarda[8], L. Villard[2], M. Goossens[1], A. Moncla[2]* 1) INSERM U841 and APHP, Hosp Mondor-CRETEIL, France; 2) INSERM U491 and APHP, Hosp La Timone-Marseille, Fr; 3) Genetics, Royal Devon and Exeter NHS Foundation Trust, UK; 4) Genetics, CHU Amiens-Nord, Fr; 5) Genetics, CH du Mans, Fr; 6) Genetics, CHU Pontchaillou, Rennes, Fr; 7) Genetics, Hosp L'Archet 2, Nice, Fr; 8) Genetics, CHU de Villeneuve, Montpellier, Fr.

Background Pitt-Hopkins syndrome (PHS) appears to be an underdiagnosed, syndromic mental retardation disorder, marked by hyperventilation and characteristic dysmorphism. PHS was shown to be caused by *de novo* heterozygous mutations of the *TCF4* gene located in 18q21. To expand the spectrum of phenotypic features associated with mutations and deletions of *TCF4*, we studied 30 unrelated patients whose phenotype overlapped PHS. **Material and methods** Among a cohort of patients without molecular abnormalities for Angelman, Mowat-Wilson, or Rett syndromes, we selected those with severe mental retardation and one or more of the flowing: PHS dysmorphologic features, microcephaly, epilepsy, and hyperventilation. *TCF4* was analysed by QMF-PCR and sequencing. Larger deletions were characterised by CGH array. All patients were karyotyped. **Results** We identified ten novel mutations in 11 patients. Cardinal manifestations were facial dysmorphism, severe developmental delay, late or absent walking, no speech, microcephaly, strabismus, and myopia. The majority had episodic hyperventilation, happy disposition and repeated mouthing of hands. **Conclusion** We report ten novel mutations, recapitulate the current knowledge of *TCF4* molecular pathology, and implement a *TCF4* database (http://www.LOVD.nl/TCF4). The large number of deletions and small deletions/insertions observed in *TCF4* leads to initiate the molecular study by QMF-PCR. No obvious departure was observed between the patients harboring point mutations and large deletions at the 18q21 locus, further supporting *TCF4* haploinsufficiency as the molecular mechanism underling PHS.

ARC syndrome - Two case reports. *I. M. FURQUIM[1], R. S. Honjo[1], G. Porta[2], A. P. R. Hirschfeld[2], J. R. Vasconcelos[2], D. R. Bertola[1], L. M. J. Albano[1], C. A. Kim[1]* 1) Genetic Unit - HC-FMUSP-São Paulo - Brazil; 2) Hepatology Unit - HC-FMUSP-São Paulo - Brazil.

ARC syndrome is an autosomal recessive multisystem rare disorder. It is characterized by neonatal cholestatic jaundice with a low GGT activity, renal tubular leak and hypotonic-related arthrogryposis. Others features reported variably are ichthyosis, mild dysmorphic signs, absent corpus callosum and recurrent febrile illness. Most patients die by the age of 7 months, but rarely some survive until 16 months with severe developmental delay. Gissen et al. (2006) characterized the molecular features of 62 individuals with ARC from 35 families, the largest cohort studied. Germline VPS33B mutations were found in 28 of 35 families (48 of 62 individuals); heterozygosity was found in the VPS33B locus in some cases of ARC, suggesting the possibility of a second ARC syndrome gene. Several different germline VPS33B mutations were restricted to specific ethnic groups. We studied two infants, both males and first child of non-consanguineous parents, born in good conditions, with normal birth weight and length. The first patient presented with bilateral hip dislocation and joint contractures in elbows and knees and the second had club foot only. Both developed neonatal cholestatic jaundice and renal tubular defects, Fanconi type, after 10 days of age. The liver biopsy revealed paucity of bile ducts and bile plugs in the first patient. He developed ichtyosis by 3 months of age and he is still alive at 5 months. The second patient died at 46 days of life due to recurrent pulmonary infections. A liver biopsy post-mortem showed severe cholestasis with hepatic regeneration and deposit of lipofuscin. Our study reinforces the high morbidity and mortality of ARC syndrome. The diagnosis of ARC syndrome should be considered in every newborn that develops cholestatic jaundice associated to any degree of joint contracture and signs of renal dysfunction. The study of the VPS33B gene is important to confirm the diagnosis and is essential for a proper genetic counselling.

Pathway-Level Analysis in a Genome Wide Association Study (GWAS) of Total Serum IgE Levels. *B. Linsay[1], C. Ober[1], D. L. Nicolae[1,2]* 1) Department of Human Genetics, University of Chicago, Chicago, IL; 2) Departments of Medicine and Statistics, University of Chicago, Chicago, IL.

GWAS are quickly becoming the standard approach for studying the genetics of complex phenotypes. However, they often lack the statistical power to yield p-values small enough to pass stringent thresholds of genome-wide significance. Here, we describe a method of statistical analysis that enhances power by considering the results of a GWAS in a biological context during analysis. This approach uses databases that catalogue biological pathways to group SNPs into smaller, biologically motivated subsets. Each subset of SNPs is examined for an excess of small p-values. To evaluate this method, we performed a GWAS of total IgE levels in 693 Hutterites using the Affymetrix GeneChip Mapping 500k Array. High quality genotypes for 295,307 SNPs with minor allele frequencies 0.05 were analyzed. SNPs were assigned to 150 pathways using the PANTHER Pathway database, and evidence for association in a pathway was quantified using a false discovery rate. Using this pathway-based approach, we identified a significant enrichment of small p-values for SNPs in or near genes involved in a serotonin signaling pathway (p=0.005 after correcting for multiple testing). While additional study is required to confirm these results, our data suggest that a pathway-based method is a useful approach for analyzing GWAS data. Supported by HL56399, HL66533, and HL85197 to C. O. and RR00055 to the University of Chicago GCRC.

CRISPLD2 assessed with multiple orofacial cleft (OFC) phenotypes in multiple ethnicities. *M. L. Marazita[1], M. E. Cooper[1], A. R. Vieira[1], A. Letra[1], R. Menezes[1], M. Mansilla[2], J. C. Murray[2], E. E. Castilla[3], I. M. Orioli[4], A. E. Czeizel[5], R. Martin[6], B. C. Chiquet[7], J. T. Hecht[7]* 1) Univ of Pittsburgh; 2) Univ of Iowa; 3) FIOCRUZ, Rio de Janeiro, Brazil; 4) Fed Univ of Rio de Janerio, Brazil; 5) Fndn Commun Ctl of Hered Dis, Budapest, Hungary; 6) Wash Univ, St. Louis; 7) Univ of Texas Med Sch, Houston.

OFC is a common birth defect with prevalence varying by ethnicity and phenotype. Significant linkage results were found at 16q24 (820 OFC families, LOD=3.55). Thus, we analyzed 4 SNPs in CRISPLD2, a 16q24.1 candidate gene () based on previously reported association in Texas families. Data: Families from Asia (287 China/Philippines), N. America (614 Iowa/Pittsburgh/St Louis/Texas), Latin America (319 Guatemala /S. American registry), and Europe (110 Madrid/Hungary/Turkey) were genotyped using Taqman assays. The Texas data included both Hispanic (82) and Caucasian (262) families. We identified 4 OFC phenotypic subgroups by family history: all affecteds with cleft lip only (CL), all with cleft palate (CP) and CL (CLP), both CL and CLP in the family (CLCLP), and at least one affected with CP only (Palate). A combination of CL, CLP and CLCLP subgroups totals CL with or without CP (CL/P). Association analyses (allelic and genotypic TDT using FBAT) were performed for all cleft and population subgroups. Results: None of the association results met the Bonferroni-adjusted alpha level for significance (0.0025). Of interest, Asian groups showed near-significant association with Palate (rs1546124 common allele G, G/G genotype; $p<0.004$). Latin American and Hispanic groups also had borderline association with Palate but with the opposite allele than in Asians (rs1546124 common C allele and C/C genotype; $p=0.03$). Non-Hispanic Caucasian North American groups showed borderline association with CLP and CL/P (rs1546124 common C allele; $p<0.03$). Conclusions: These results continue to suggest that CRISPLD2 may be involved in risk of OFC. Further genotyping and expression studies are necessary to further characterize the involvement of CRISPLD2 and to identify the causal variant(s). DE09886, DE08559, DE016148, DE016930, DE016215.

A role for genetic variation at HOMER2 in schizophrenia: further evidence from Irish and other European populations. *W. P. Gilks, E. H. Allott, M. Gill, A. P. Corvin, D. W. Morris, The International Schizophrenia Consortium*
Neuropsychiatric Genetics, Department of Psychiatry and Institute for Molecular Medicine, Trinity College Dublin, Ireland.

SCHIZOPHRENIA (SZ) is a complex disorder of uncertain aetiology but which may involve dysfunction at glutamatergic synapses of the brain. Based-on linkage and proteomic data we identified HOMER2 (OMIM 604799) as a plausible candidate gene for SZ. Homer2 proteins localise to the post- synaptic density linking glutamate receptors with the cytoskeleton. We previously reported evidence of association at HOMER2 in a sample of 375 cases and 812 cases from Ireland (Gilks et al. 2007 World Congress in Psychiatric Genetics XV). The best result was an allelic association at rs869498 ($p=0.016$, OR 1.39). Three other SNPs (rs1817658, rs12913501 and rs2306428) were also associated ($p<0.05$, OR1.2) and independent of LD (r2 cut-off 0.7). The strongest haplotypes were (rs2306428- rs12913501, $p=9x10^{-4}$ and rs869498- rs1871658, $p=0.0025$). The International Schizophrenia Consortium have conducted a genome-wide association study of schizophrenia using 3,380 cases and 3,593 controls from Europe (Affymetrix 5.0 and 6.0 platforms). Across HOMER2, 44 SNPs were genotyped of which 11 were associated with disease status at $p<0.05$. Of our four previous LD-independent associations, two (at rs2306428 and rs869498) were reproduced by proxy (rs17158194, $p=0.001$, OR 1.15 and rs17158155, $p=0.02$, OR 1.27 respectively). We have also found evidence for association in this dataset at genes regulated by HOMER2 . These data support a role for HOMER2 in SZ susceptibility and further genetic and functional studies are warranted to investigate molecular pathways involving HOMER2 in SZ.

Regulatory mechanisms of the expression of WNK1, a serine-threonine kinase involved in Familial Hyperkaliemic Hypertension (FHHt). *E. E. Elvira-Matelot[1,2,3], C. D. Delaloy[1,2,3], X. Z. Zhou[1,2,3], X. J. Jeunemaitre[1,2,3], J. H. Hadchouel[1,2,3]* 1) INSERM U772; 2) College de France; 3) Universite Paris Descartes.

The genetic study of Familial Hyperkalaemic Hypertension (FHHt), a rare form of hypertension, revealed a new mechanism of blood pressure regulation, through the identification of WNK1 and WNK4. These kinases, expressed in the renal distal tubule, play a complex role in renal ionic transport regulation. WNK1 and WNK4 mutations were found in only two of the 34 FHHt families recruited by our group. WNK1 gives rise to long isoforms ubiquitously expressed (L-WNK1) from the proximal promoters P1 and P2, and to a short renal isoform (KS-WNK1) from the promoter rP. FHHt mutations are large deletions in the first intron 1 of WNK1. We showed that these deletions lead to an overexpression of L-and KS-WNK1 in the DCT and to a generalized ectopic expression of KS-WNK1. These data suggest the presence in intron 1 of one or several repressor and/or insulator elements. Our first objective was to identify and characterize these different elements. Five conserved non-coding sequences (C1-5) were identified in intron 1 by cross-species sequence comparison. Transfection assays showed that C1 represses rP transcriptional activity while C5 plays the role of an insulator. FHHt could therefore be the consequence of the deletion of at least one repressor and one insulator. Our second objective was to study more generally the different mechanisms regulating the expression of WNK1 in order to identify other sequences potentially mutated in the remaining FHHt patients. In silico analysis identified in the 3UTR region of WNK1 target sequences for micro-RNAs miR-192 and miR-215. We showed in vitro that these miRs could negatively regulate WNK1 expression at the post-transcriptional level. We also showed that potassium and sodium intake modulates the expression of WNK1 and these miRs. We are now studying the detailed miRs expression pattern and their contribution to WNK1 regulation in vivo.

Association-study of polymorphisms in the GAD2 gene-region in patients with anxiety-disorder, unipolar depression and healthy controls. *P. G. Unschuld, M. Ising, M. Specht, A. Erhardt, S. Ripke, A. Heck, S. Kloiber, T. Brueckl, B. Müller-Myhsok, F. Holsboer, E. B. Binder* Max Planck Institute of Psychiatry, Kraepelinstr.2-10, 80804 München, Germany.

Glutamate decarboxylase (GAD) is the rate limiting enzyme for conversion of glutamic acid to gamma-aminobutyric acid (GABA). GABA is the major inhibitory neurotransmitter in the vertebral central nervous system and a promising target for anxiety medications. The GAD 65kDa isoform is encoded by the gene GAD2 and is mainly expressed in synaptic terminals. It serves as an apoenzyme, which shows enhanced availability in situations of stress, responding to short term demands for GABA. We analyzed 18 single nucleotide polymorphisms (SNPs) in the GAD2-gene region and derived nine tagging SNPs. These nine SNPs were evaluated for associations with psychiatric diagnosis and behavioral inhibition (BI) derived from the personality traits neuroticism and extraversion as defined by the Eysenck Personality Questionaire (EPQ). A total of 268 patients with anxiety disorder (AD) and 537 with unipolar depression (MD) and 545 matched healthy controls, were analyzed for disease associations. 413 of these patients and 537 healthy controls were further analyzed for associations with BI. We observe significant associations for five tag-SNPs with behavioral inhibition in the AD- and control samples as well as one additional case-control association in the MD-sample. One associated tagging-SNP lies within a 16kb linkage disequilibrium-block, including putative 5 GAD2-promotor-elements as well as the 3 end of the gene MYO3A. Using Hap-map expression data we could show that BI-associated SNPs appear to be associated with differences in MYO3A- but not GAD2 lymphocyte-mRNA expression levels. These results support earlier studies that suggest associations of polymorphisms within the GAD2 locus with anxiety and affective disorders. However, data from expression studies may indicate that these polymorphisms could tag functional effects on the neighboring gene MYO3A, which is also expressed in the brain, including the cingulate cortex and the amygdala.

Comparison of genotyping consistency between genomic and whole-genome amplified DNA using the Illumina GoldenGate and Infinium-II assays. *P. Hoffmann[1,2], S. Herms[1], M. Alblas[1], K. Kemmerling[1], T. W. Mühleisen[1], M. M. Nöthen[1,2], S. Cichon[1,2]* 1) Department of Genomics, Life & Brain Center, University of Bonn, Bonn, Germany; 2) Institute of Human Genetics, University of Bonn, Bonn, Germany.

High-throughput SNP genotyping has become an important research strategy in human genetics. Although most genotyping assays require minimal amounts of DNA, repeated use often leads to depletion of the mostly irreplaceable samples. To address this problem whole-genome amplification technologies have been developed in the last years and are meanwhile commercially available. Albeit the amplification seems to be mostly successful, it is controversially discussed whether the whole genome amplified DNA (wgaDNA) represents an exact copy of the genomic DNA (gDNA) template. In the present study, we aimed to assess the genotyping consistency between 45 wgaDNAs (generated using the REPLI-g DNA Amplification Kit, Qiagen, Hilden) and their corresponding gDNA samples. The gDNAs were of different age and quality. In a first step we compared genotype consistency between wga and gDNA. in 20 high quality sample pairs genotyped using Illuminas HumanHap550V3.0 BeadChips (565.000 SNPs) and 25 sample pairs of different DNA quality genotyped for 384 SNPs using Illuminas GoldenGate assays. All samples genotyped on the BeadChips performed well, with average call rates of >99%. The average consistency between gDNA and wgaDNA was 99.99% when comparing SNPs successfully genotyped in the corresponding samples. Of the 25 sample pairs genotyped with GoldenGate assays, 22 performed well with average call rates >99% (gDNA) and >98% (wgaDNA). Genotype consistency between wga and gDNA was 100% for all SNPs successfully genotyped in both corresponding samples. The remaining 3 sample pairs showed noticeably worse results with an average genotype call frequency of 99.8% (gDNA) versus 60.1% (wgaDNA) and a genotype consistency of only 89%. Possible explanations for the observed discrepancies are age of gDNA, extraction method as well as presence of unknown inhibitors interfering with the amplification process.

Genomic rearrangements encompassing and upstream of *SHOX* identified using MLPA and a newly developed qPCR assay. *B. D'haene[1], M. Craen[2], J. De Schepper[2, 5], K. Devriendt[3], G. Massa[4], J. P. Fryns[3], K. Keymolen[5], B. A. Bejjani[6], A. de Klein[7], E. M. De Jong[7], B. Loeys[1], J. G. Leroy[1], G. Matthijs[3], L. Mutesa[8], K. Segers[8], P. Willems[9], N. Van der AA[10], A. De Paepe[1], G. Mortier[1], E. De Baere[1]* 1) Ctr Medical Genetics, Ghent Univ Hosp, Ghent, Belgium; 2) Dpt of Pediatrics, Ghent University Hospital, Belgium; 3) Ctr for Medical Genetics, University of Leuven, Belgium; 4) Virga Jesse Hospital, Hasselt, Belgium; 5) Ctr for Medical Genetics, Free University of Brussels, Belgium; 6) Signature Genomic Laboratories, WA, USA; 7) Clinical Genetics, Erasmus MC, Netherlands; 8) Ctr for Human Genetics, University of Liège, Belgium; 9) Gendia, Antwerp, Belgium; 10) Ctr for Human Genetics, University of Antwerp, Belgium.

The human *SHOX* gene is the only known disease gene within the pseudoautosomal region 1 (PAR1) of the sex chromosomes. Haploinsufficiency of this gene due to intragenic mutations, total gene deletions or microdeletions upstream of *SHOX* has been reported in patients with idiopathic short stature (ISS) and Leri-Weill dyschondrosteosis (LWD). We screened 161 patients with ISS, LWD and some other indications for copy number variations of the *SHOX* region using MLPA (P018, P018B and P018C, MRC-Holland) and/or quantitative PCR (qPCR) with 11 amplicons for the *SHOX* gene and PAR1 region. Using MLPA we identified a copy number change in 17 patients, including 10 *SHOX* deletions, 4 duplications and 3 upstream deletions. Second, a new qPCR assay for the *SHOX* region was developed and validated. Using this we identified 5 new rearrangements, including 3 deletions and 2 duplications of *SHOX*. Overall, a genomic rearrangement in the *SHOX* region was found in 14% (22/161) of the probands. In this study we designed and validated a new qPCR assay for *SHOX* and the PAR1 region. This technique is very flexible in terms of adding new amplicons, and does not require parental DNA as compared to microsatellite analysis. Our assay is an alternative strategy for the identification of genomic rearrangements in LWD and ISS that can be applied in both research and clinical settings.

Analysis of polymorphic variations in genes of the dopamine and serotonin pathways with iloperidone efficacy in the treatment of patients with schizophrenia. *S. Volpi, K. Mack, C. Lavedan* Vanda Pharmaceuticals, Inc, Rockville, MD.

Schizophrenia is a chronic, severe, and disabling disorder that affects about 1% of the population worldwide. Molecular components of the dopaminergic and serotoninergic systems are believed to play an important role in the pathophysiology of schizophrenia and the mechanisms of action of antipsychotics. Iloperidone is a novel mixed D2/5-HT2 antagonist with significant affinity for the dopamine D2 *(DRD2)* and D3 *(DRD3)* and the serotonin 5-HT2A *(HTR2A)* receptors. It has moderate affinity for the D4 *(DRD4)* receptor and low affinity for the serotonin 5-HT2C *(HTR2C)* and 5-HT1A *(HTR1A)* receptors. In an ongoing effort to identify patients with schizophrenia who could benefit the most from iloperidone treatment, a pharmacogenetic analysis was conducted in a phase III clinical trial to discover genetic markers predictive of response. We investigated the effect of various DNA polymorphisms in genes suspected to play a role in schizophrenia and/or antipsychotic treatment response on iloperidone efficacy: *DRD2* (-241A/G, -141C Ins/Del, His313Cys), *DRD3* (Ser9Gly), *DRD4* (-521C/T), *HTR1A* (-1019C/G), *HTR2A* (102T/C) and HTR2C (-759C/T). A general linear model analysis of variance was performed by genotype on improvement of symptoms assessed by the Positive and Negative Syndrome Scale (PANSS) Total score. None of the polymorphisms evaluated were found to be significantly associated with iloperidone efficacy. These results suggest that the genetic variations tested in this study do not contribute significantly to inter-individual differences in the therapeutic efficacy of iloperidone; genetic markers of response for a particular drug may not be applicable to the whole class of antipsychotics, reflecting differences in receptor binding profiles and in chemical and metabolic characteristics. In addition, these findings support the application of pharmacogenetics to differentiate medication options and improve individualized treatments for schizophrenia.

Gene-gene interaction between Parkin and NOS2A in Parkinson disease. *L. Wang, G. Mayhew, A. Burt, M. Slifer, G. Wang, S. Zuchner, W. Scott, J. Vance, E. Martin* Miami Inst Human Gen, Univ Miami,Miller Sch Med, Miami, FL.

Exonal deletions and duplications (del/dup) in Parkin cause autosomal recessive early-onset Parkinson disease (PD). A synonymous single nucleotide polymorphism (SNP), rs1060826, in the NOS2A gene has shown association with sporadic PD in Finnish, French and United States populations. The protein product of NOS2A, inducible nitric oxide synthase, is responsible for massive production of nitric oxide (NO). Excessive NO leads to S-nitrosylation of parkin, the post-translational modification which inhibits parkins ubiquitin E3 ligase activity and leads to abnormal accumulation of misfolded proteins Therefore, we hypothesize that variants in Parkin and NOS2A interact in their contribution to PD risk. To test this hypothesis we investigated gene-gene interaction between polymorphisms in NOS2A and del/dup of Parkin in a sample of 757 PD probands (drawn from our family sample) and 176 unaffected controls. Parkin del/dup was measured using real time PCR. For this analysis, we scored an individual as a Parkin mutation carrier if they carry any del/dup in the gene. We studied 18 HapMap tagSNPs in NOS2A previously genotyped in our entire family-based samples. We conducted case-only analysis as our primary gene-gene approach due to the low frequency of Parkin mutations in controls. We found evidence of interaction between Parkin del/dup status and two synonymous SNPs in NOS2A (rs1137933, p=0.044 and rs1060826, p=0.024). Inspection of NOS2A genotype frequencies shows that NOS2A genotypes in controls are similar to PD probands with Parkin del/dup (p=0.718) but are significantly different from PD probands without a Parkin del/dup (p=0.033). These results suggest that NOS2A variants exert their effects only in individuals without a Parkin del/dup. This could indicate that the variants in NOS2A increase PD risk through altering the function of wild type parkin by S-nitrosylation of the protein. Our study highlights the interaction between NOS2A and parkin in PD. This interaction has important implications for future analysis of these genes and our understanding of the molecular mechanism underlying the etiology of PD.

Large deletions account for a significant fraction of mutations in Marfan syndrome. *B. M. Rhode[1], L. Föhse[1], M. Stuhrmann[1], D. Steinemann[2], T. Hellwig[1], A. Hein[1], J. Schmidtke[1], M. Arslan-Kirchner[1]* 1) Institute of Human Genetics, Hannover Medical School, Hannover, Germany; 2) Institute of Cell and Molecular Pathology, Hannover Medical School, Hannover, Germany.

Marfan syndrome (MFS, OMIM 154700) is caused by heterozygous mutations in the *FBN1* gene. Cardinal features involve the ocular, cardiovascular and skeletal systems. Overlapping syndromes such as the Loeys-Dietz syndrome (LDS1A, OMIM 609192; LDS1B, OMIM 610168; LDS2A, OMIM 608967; LDS2B, OMIM 610380) are caused by mutations in the *TGFBR1* or *TGFBR2* genes and show either a Marfan-like phenotype (LDS2A, LDS2B) or a more severe phenotype characterized by arterial tortuosity and aneurysms as well as craniofacial involvement (LDS1A, LDS1B).

We screened over 600 patients with suspected Marfan syndrome or Loeys-Dietz syndrome for a mutation in the *FBN1* and/or the *TGFBR1* and *TGFBR2* genes by direct sequencing and present a selection of previously not described mutations in these genes. The detection rate of mutations in the *FBN1* gene in patients with Marfan syndrome lies between 70 to 90%, while sequencing of the *TGFBR1* and *TGFBR2* genes adds another 5 to 10%. In 91 patients, we applied MLPA (multiplex ligation-dependent probe amplification) to screen for large deletions in the *FBN1* and *TGFBR2* genes. All patients were tested negative for a mutation in *FBN1* by sequencing. 60 of these patients were also tested for a mutation in the *TGFBR1* and *TGFBR2* genes by sequencing, and were found to be negative for such a mutation. To date, we have identified six large deletions in the *FBN1* gene (exons 24-26, 50-54, 55-58, 58-63, 1-65, and 6-65). Breakpoints of the deletions were either determined by long-range PCR techniques followed by sequencing or by high resolution array CGH. Patients phenotypes range from a classical syndrome spectrum to a severe neonatal Marfan syndrome. The detection rate of deletions in this pre-screened patient group is 6.7%. Our MFS patient cohort thus harbors the highest *FBN1* deletion rate reported so far. Our data suggest that the overall mutation detection rate in patients with Marfan syndrome can be increased significantly by MLPA analysis.

Tissue Specific Genetic Variation and Vulnerability To Disease: The BAK Gene and Abdominal Aortic Aneurysms. *M. Schweitzer[1,2,3], L. E. Chalifour[1,2,3], B. Gottlieb[2,4]* 1) Sir Mortimer B. Davis -Jewish General Hospital, Montreal, PQ, Canada; 2) Lady Davis Institute for Medical Research, Montreal, PQ, Canada; 3) Dept., Medicine, McGill University, Montreal, PQ, Canada; 4) Dept., Human Genetics, McGill University, Montreal, PQ, Canada.

Abdominal aortic aneurysm (AAA) is a multifactorial disease with both genetic and environmental factors playing crucial roles in disease ontogeny. We sought to examine why the abdominal aorta is more susceptible (80 percent) to aneurysms than other arteries. To examine the role of genetics, we studied sequence variation in the BAK gene (*BAK*) that codes for an apoptotic-promoting protein, as apoptosis activation has been linked to AAA development and progression. We sequenced *BAK* cDNA from 31 patients as well as from non-diseased individuals and compared the *BAK* abdominal aorta cDNA sequence to *BAK* genomic sequence obtained from matching blood samples. Our results revealed specific *BAK* alterations (SNPs) in both healthy abdominal aortic tissue as well as aneurysms, which were not present in the matching blood samples. These same *BAK* SNPs have been reported, though rarely, in reference *BAK* DNA sequences. Our results suggest that in diseases such as AAA, tissue disease susceptibility is related to pre-existing genetic alterations that are selected for in specific tissues. Based on this and other similar observations, we propose a new hypothesis, for the origin of tissue-specific diseases that can help explain the role of both environmental and genetic factors. Our hypothesis postulates that mutant genes preexist in minority forms within non-diseased tissues and are selected for, when tissue and cellular conditions change. We believe that such a theory can better help explain the ontogeny not only of cardiovascular diseases, such as AAA, but other multifactorial diseases as well.

Microarray analysis of candidate genes in a child with 18p subtelomeric deletion and atypical early onset of dystonia and neuroregression. *E. Cole, J. Y. Lee, S. Li, S. E. Palmer* Dept. of Pediatrics, Section of Genetics, Univ. of Oklahoma Health Sciences Center, Oklahoma City, OK.

18p- syndrome, like most deletion syndromes, is usually considered a static disorder. However, a few patients with large 18p deletions on karyotype have developed dystonia, with onset from adolescence to adulthood. All deletions included distal 18p, some confirmed by subtelomere FISH. Severity of dystonia varied from mild to mostly disabling. We report a female with normal karyotype and smaller 18p deletion diagnosed by subtelomeric FISH. Presenting features were developmental delay, seizures, hypotonia, and mild dysmorphic features. Before age 3 years, she developed dystonic and choreoathetotic movements much earlier than reported; this was accompanied by atypical neuroregression of all milestones. Work-up of the neurologic features has been non-diagnostic. The etiology of dystonia in 18p- patients was reconsidered. Whole genome oligo array CGH showed this patients deletion was estimated at 2.7 Mb involving p terminus to p13.11. This includes approximately 21 known genes; all but one have no known association to human disease, although several have proposed roles in neurologic function. The deletion includes most of the *DYT7* locus reported to be linked to an autosomal dominant late-onset distal focal dystonia, with a phenotype much milder than the dystonia reported in most 18p- patients. Several of the case reports of 18p- with dystonia have shown their larger deletions also include the *DYT7* locus. The 18p dystonia locus, *DTY15*, is more proximal and not deleted here. This patients much smaller deletion supports the hypothesis that a gene for disabling dystonia in 18p- maps just to the subtelomeric region; whether *DYT7* is the same gene remains unclear due to its milder phenotype. The earlier onset of dystonia with additional atypical neuroregression in this child raises the question of the exact frequency, severity, type, and age range of onset of movement disorder in the natural history of these patients. The identification of the deleted genes now localized to a much smaller ~2.7 Mb region provides the basis for additional studies of candidate genes for this dystonia.

Genomic Approaches to Identifying the Molecular Basis of Morphological Variation in Avian Beaks. *K. Powder[1], S. Brugmann[2], J. Helms[2], M. Lovett[1]* 1) Washington Univ, St Louis, MO; 2) Stanford Univ, Stanford, CA.

Vertebrates, particularly birds, exhibit remarkable facial variation. Previous work by JH used cell grafting to show that cranial neural crest (NC) cells transplanted between embryonic quails and ducks developed facial features reminiscent of the donor species. This showed that NC cells provide facial patterning information, but left unresolved which specific genes or pathways differ between these species. Here we describe the molecular signatures, pathways and transcription factor (TF) gene expression changes between NC populations of the presumptive beak in three bird species (chicken, duck, and quail) at two developmental stages. We observed that prior to morphological distinctions between the species, NC cells have established a species-specific gene expression profile that is largely unchanged even to stages when morphological differences are evident. 335 genes were differentially expressed (>2-fold with p-values < 0.05) between the three species. The majority of these genes (145) and those with the largest fold changes were differentially expressed in the duck compared to the chicken and quail. Known developmental pathways such as TGF and Fgf, plus an additional 24 previously identified regulators of craniofacial development, exhibited large changes between species. We also observed large changes in 74 TF genes with, as yet, unknown functions. Duck NC cells show a dramatic 20-fold up-regulation of the WNT signaling components *Dkk2*, *Fzd1*, *Wnt1*, and *Wnt10b*, and a 10-fold up-regulation of the putative WNT interactors *Klhl12* and *Tbx20*. The *Calmodulin1* and *Calmodulin2* genes, previously shown to contribute to elongated beak shapes in Darwins finches, are down-regulated in the duck relative to the other two species. We confirmed microarray observations by RNA in situs that also revealed differences in spatial patterns of expression. We have further investigated the pathways of beak development by knocking down differentially expressed TFs using siRNAs in NC cell populations. By conducting gene expression analysis on knockdowns, we have begun to identify novel interactions between TF genes involved in vertebrate facial development.

New analytic methods to detect a polygenic or locus specific genetic effect from large families adjusting for non-genetic environmental factors: single and multiple trait cases. *H. Lee*[1], *M. C. Paik*[2], *J. P. Krischer*[1] 1) Pediatrics Epidemiology Center, University of South Florida, Tampa, FL; 2) Department of Biostatistics, Columbia University, New York, NY10032, U.S.A.

New analytic methods for quantitative genetics are proposed using regression models for correlation parameters, which is proper for large family studies. We interpret conventional heritabilities as regression parameters adjusting for subject or pair specific non-genetic differences. This approach provides statistical tests robust to normality assumption on quantitative traits. For single trait analysis, we implement the efficient three generalized estimating equations (efficient 3GEEs) proposed by Lee et al.(2008a), and for multiple trait analysis, the method for multivariate familial correlation analysis proposed by Lee et al.(2008b) is applied. The example data in SOLAR manual were used to illustrate our proposed methods.

Genetic testing for Leber Congenital Amaurosis (LCA): a 3-year experience. *F. Coppieters[1], T. de Ravel[2], I. Casteels[3], F. Meire[4], N. Van Regemorter[5], S. De Jaegere[1], A. De Paepe[1], P. Coucke[1], B. P. Leroy[1,6], E. De Baere[1]* 1) Ctr for Medical Genetics Ghent, Ghent University Hospital, Ghent, Belgium; 2) Ctr for Human Genetics, Leuven University Hospitals, Leuven, Belgium; 3) Dept of Ophthalmology, Leuven University Hospitals, Leuven, Belgium; 4) HUDERF, Hopital Des Enfants Reine Fabiola, Brussels, Belgium; 5) Centre de Génétique de Bruxelles, Free University of Brussels, Brussels, Belgium; 6) Dept of Ophthalmology, Ghent University Hospital, Ghent, Belgium.

LCA is genetically highly heterogeneous with an involvement of large disease genes, which hamper genetic testing. The purpose of this study was to determine the prevalence of mutations in 6 common LCA genes in 98 LCA patients, mainly of Belgian origin, in order to optimize a genetic screening strategy for LCA. First, LCA chip screening revealed a mutation in 34% of all patients. Second, direct sequencing of *AIPL1*, *CRB1*, *CRX*, *GUCY2D*, and *RPE65* in chip-negative tested patients revealed causal mutations in 3%. Third, we performed targeted mutation analysis of *CEP290* mutation c.2991+1655A>G. We found this mutation in both homozygous (2/98) and heterozygous (16/98) state. A second mutation was identified through sequencing of the total coding region. Subsequently, the remaining patients were screened for 4 additional recurrent *CEP290* mutations. The allele frequencies of the most common mutations p.Lys1575X and c.[3310-1G>A;3310C>A] were respectively 13% and 9%. Finally, sequencing of the total coding region of *CEP290* is being performed in the remaining cases. So far, this revealed a homozygous mutation in one case. In addition, we identified *RDH12* mutations in 2 families with early-onset retinal dystrophy, and *CEP290* mutations in 3 families with Senior-Loken syndrome. In conclusion, we found both disease causing mutations in 59% of all LCA patients (22% in *CEP290*; 18% in *CRB1*; 8% in *RPE65*; 6% in *GUCY2D*; 3% in *AIPL1* and 2% in *CRX*). A combined genetic testing strategy consisting of LCA chip analysis and targeted mutation screening of 3 recurrent *CEP290* mutations represented an efficient first-pass screening, revealing causal mutations in 55% of our LCA population.

An in vivo unbiased screen for enhancer activity using lentivector-mediated transgenesis. *M. Friedli[1,5], I. Barde[2,5], C. Attanasio[1,5], A. Quazzola[2,5], S. Verp[2.5], M. Arcangeli[1,5], F. Spitz[3,4,5], J. Zakany[3,5], D. Duboule[2,3,5], D. Trono[2,5], S. E. Antonarakis[1,5]* 1) Genetic Med & Development,Univ Geneva,Geneva,CH; 2) School of Life Sciences,EPFL,Lausanne,CH; 3) Department of Zoology and Animal Biology, University of Geneva, CH; 4) EMBL,Heidelberg,Germany; 5) National Research Centre Frontiers in Genetics.

Finding sequences that control spatial and temporal expression of genes is important to understand genome function. Here, we present an in vivo screen for enhancers in a contiguous 200 kb DNA fragment using lentivector-mediated transgenesis. Previous studies have used evolutionary conservation as an indicator of regulatory potential, but increasing evidence suggests that this criterion systematically overlooks functional sequences. We thus designed our study without any bias towards a particular sequence feature. We chose a mouse BAC corresponding to a region of Hsa21 because it contains the olig genes that are expressed specifically in the CNS. In order to screen systematically for enhancer activity, we generated a library of 134 overlapping clones in a LacZ reporter lentiviral construct containing a minimal promoter. We generated lentivectors individually for each segment and injected them in pools of 10 or 20 in mouse oocytes. LacZ staining was performed on E11 embryos to identify expression patterns. The seven pools tested yielded 70 of 301 LacZ positive embryos with ~2.5 transgenes per embryo. Several fragments of the 64 assessed were identified that potentially contain gene expression regulators. Individual injection of candidates confirmed 2 elements (one evolutionary conserved) as tissue specific enhancers with stainings in the spinal chord/brain and trigeminal ganglion compatible with olig expression. For one element, orthologous human and chicken sequences were injected and showed similar expression patterns, demonstrating that these activities are conserved in other species. ISH confirmed that these elements likely contribute to the expression pattern of olig genes. The method could be scaled up to cover large chromosomal regions, and determine what fraction of the constrained and non-constrained genome has regulatory potential.

Rare copy number variants reveal novel candidate genes for schizophrenia. *T. Vrijenhoek[1], J. E. Buizer-Voskamp[2, 3], I. van der Stelt[1], R. S. Kahn[4], C. Sabatti[5], R. A. Ophoff[3, 6], J. A. Veltman[1]* 1) Department of Human Genetics, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands; 2) Rudolf Magnus Institute of Neuroscience, Department of Psychiatry, University Medical Centre Utrecht, Utrecht, The Netherlands; 3) Complex Genetics Section, DBG-Department of Medical Genetics, University Medical Centre Utrecht, Utrecht, The Netherlands; 4) Division of Neurosciences, Department of Psychiatry, University Medical Centre Utrecht, Utrecht, The Netherlands; 5) Department of Human Genetics, University of California, Los Angeles, USA; 6) Centre for Neurobehavioral Genetics, University of California, Los Angeles, USA.

Schizophrenia is severe psychiatric disease with complex etiology, affecting approximately one percent of the general population. Most genetic studies so far focused on disease association with variation at the SNP level, but it has become apparent that genomic copy number variants (CNVs) may be involved in disease susceptibility as well. To assess the role of CNVs in schizophrenia, we screened 54 patients with deficit schizophrenia with Affymetrix GeneChip© 250K SNP arrays. We identified in total 90 CNVs, 76 of which have been reported previously to be present in the general population. Among the genes disrupted by the 14 rare CNVs are MYT1L, CTNND2, NRXN1 and ASTN2, three of which have never been associated before with schizophrenia. These genes play an important role in neuronal function, including axon guidance, neuron adhesion and neuron migration, and they are highly expressed in the brain. We further studied these regions in a cohort of 752 patients and 706 controls from The Netherlands and identified an additional 7 patients and 1 control subject with CNVs affecting these same loci (Combined P = 0.007; OR = 9.7). Our study supports the role of rare variants involved in schizophrenia susceptibility and identifies three novel candidate genes important in brain development.

Optimal methods to map quantitative trait loci using extreme trait values. *D. Covarrubias[1], S. M. Leal[2]* 1) Department of Statistics, Rice University, Houston, TX; 2) Molecular and Human Genetics, Baylor College Med, Houston, TX.

For mapping quantitative trait loci, one study design is to select a subset of individuals with extreme high and low quantitative trait (QT) values. This study design has been implemented to increase power while reducing the number of individuals who are either genotyped or sequenced. This study design was recently used to identify genes for HDL cholesterol and cardiovascular disease. There are several frameworks for extreme QT sampling which can be used: phenotyping study subjects until a set sample size of individuals meeting a predefined QT criterion are obtained, or using an existing sample and analyzing only a subset of individuals based upon a QT threshold. For this study, we examined the latter sampling framework for a variety of sampling proportions, allele frequencies and total sample sizes. Thresholds which optimize power were determined when the QT is dichotomized. Additionally, type I and II error was evaluated for ANOVA, linear regression, Cochran-Armitage test for trend, and ^{2} test of independence. For the analysis of QTs the highest power is obtained when the entire sample is analyzed and the power decreases with increasing extreme QT sampling. When the QT is dichotomized, using only those individuals with the highest and lowest QT values, the optimal power is obtained when ~50% of the total sample is analyzed. For this situation, the ^{2} test is most powerful when the underlying genetic model is either recessive or dominant; however for an additive model the Cochran-Armitage test for trend is the most powerful. If thresholds of > 25% are used for sampling, linear regression is the most powerful test among those evaluated however ANOVA may have superior power in some situations. Additionally for all the test which were assessed type I error was well controlled.

Grammatical Evolution Neural Networks (GENN) to detect gene-gene and gene-environment interactions that predict quantitative traits. *N. E. Hardison, A. A. Motsinger-Reif* Bioinformatics Research Center, Department of Statistics, North Carolina State University, Raleigh, NC.

The advent of increasingly efficient collection of genetic information has resulted in a great insurgence of data, leading to a need for novel methods of analysis beyond that of traditional parametric statistical approaches. Recently, we introduced a Grammatical Evolution Neural Network (GENN), a machine-learning approach to detect gene-gene or gene-environment interactions, also known as epistasis, in high dimensional genetic epidemiological data. GENN has previously been shown to be highly successful in a range of simulated and real datasets, but has been limited to case-control study designs. In the current study, we extend the GENN method to evaluate continuous traits, by incorporating an r-squared fitness function into the evolutionary process, and conduct a power study in a wide range of simulated data. We demonstrate that the GENN method is capable of detecting both single-locus models and purely epistatic two-locus models. Additionally, we demonstrate the timing of GENN analysis is amenable to large-scale genotype data. GENN software is open source, and available from the authors on request.

Conditional APL-OSA for evaluation of multiple risk variants. *X. Lou[1,2], S. Schmidt[1], E. Hauser[1]* 1) Center for Human Genetics, Duke University, Durham, NC; 2) Bioinformatics Research Center, North Carolina State University, NC.

Genetic models applied in association studies of complex disease often assume a single susceptibility variant in a small region or in a gene. However, once susceptibility genes are identified, it is often the case that multiple variants can individually change the functional qualities of a gene. Thus the models under consideration must include allelic heterogeneity models along with the single variant model. However it is difficult to tell whether multiple associated SNPs are reporting on a single disease risk variant or on multiple risk variants. We performed a simulation study to evaluate whether APL-OSA, a family-based association method for identifying genetic heterogeneity using a trait related covariate, could be applied to family-specific association evidence itself to identify allelic heterogeneity. We used the simulation package SIMLA to generate models from a two disease locus system. We simulated a common disease with prevalence of 0.20 with two marker haplotypes independently associated with two recessive disease loci, each having a GRR of 3.0 and risk allele frequency of 0.15, with different levels of LD between (i) the disease and marker alleles and (ii) between the marker alleles. The LD ranged from r2=0.18 to r2=1.0. We applied APL-OSA conditioning on the family-specific association statistics at other markers as covariates, with the goal of identifying subsets of families preferentially segregating one of the disease alleles. Simulation under the null hypothesis of no association showed that APL-OSA using family-specific APL statistics as covariates at other markers maintained the appropriate 0.05 type I error rate. Simulation studies under the alternative models showed excellent power (>0.95) to discriminate among the first and second risk locus, even at the lowest r2 of 0.18. Thus, a conditional test such as APL-OSA is a powerful tool for evaluating the presence of multiple risk variants.

Comparison between automated FISH analysis and RQ-PCR as monitoring tools for minimal residual disease (MRD) in CML. *G. Calabrese[1,2,3], D. Fantasia[1], F. Pompetti[4], R. DiGianfilippo[3], D. Romagno[1], E. Morizio[1], P. Guanciali-Franchi[1,3], M. Alfonsi[1,3], C. Nuzzi[3], R. DiLorenzo[5], A. Iacone[4], G. Fioritoni[5], G. Palka[1,3]* 1) Scienze Biomed/Genetica Medica, Univ G D'Annunzio, Chieti, Italy; 2) Center for Ageing, DAnnunzio Foundation, Chieti, Italy; 3) Servizio di Genetica Umana; 4) Dip. Med Trasfusionale; 5) Dip. Ematologia, Ospedale di Pescara, Italy.

To compare RQ-PCR method, to date the gold standard approach for MRD monitoring in CML, with a novel FISH analysis approach based on a fully automated FISH slide scanner and image analyzer (Duet BioView, Israel), we investigated 51 CML patients in clinical and cytogenetic remission following imatinib therapy (IM; 44 patients), or hematopoietic stem cell transplantation (7 patients). Ninety samples, 75 bone marrow aspirates and 15 peripheral blood withdrawals, were tested with both RQ-PCR using TaqMan protocol (Applied BioSystems, USA), and FISH slide scanner by scoring 1600-4500 cells for BCR-ABL rearrangement using a dual-fusion FISH probe (Kreatech, DK). Leukemic cell levels in the samples were arbitrarily grouped in 3 classes: 1%; 0.99%-0.04%; and <0.04% of scored cells. FISH/RQ-PCR concordance was 100% for class 1%, 96% for class 0.99%-0.04%, and 84% for class <0.04% leukemic cells. Samples with FISH/RQ-PCR discordant results showed leukemic cells as evidenced by FISH close to the detection limit of FISH procedure (i.e. 0.04%) with <0.01% Bcr-Abl transcript level and molecular remission in the following 18 months of treatment. In 2 patients FISH unravelled 3/1900 (0.16%), and 8/3800 (0.21%) leukemic cells carrying 2 copies of BCR-ABL fusion, i.e. double Ph, which were undistinguishable from those with a single copy of BCR-ABL rearrangement as by RQ-PCR. IM dose escalation (800mg/day) resulted in disappearance of double BCR-ABL leukemic cells, which are still absent 30 and 38 months from high-dose therapy start, respectively. In conclusion, automated FISH results largely overlapped with RQ-PCR data. Furthermore, double Ph-positive cells could also be early recognized by automated FISH analysis allowing appropriate therapy protocol modification.

Fundamentals of Allele Flipping in Association Studies. *G. Clarke* Dept Bioinformatics, Oxford Univ, Oxford, United Kingdom.

The Fundamentals of Allele Flipping in Association Studies G.M.Clarke(1), L.R.Cardon(2) (1) Wellcome Trust Centre for Human Genetics, University of Oxford, UK (2) GlaxoSmithKline, Philadelphia, USA Replication of initial findings in an independent sample is the gold standard for confirmation of a genuine disease-marker association. Replication is generally taken to mean association of the same SNP with the same direction of effect. Reports of allele flips, where an initial study finds an allele to be protective but a follow-up study finds it to be causative, are increasing. It is therefore of practical interest to determine when an allele flip is genuine, that is, when a high risk allele in an original study is, in fact, the low risk allele in a replication study. Allelic heterogeneity, locus heterogeneity, variation in environmental exposures and population differences are all examples of factors that can combine to create scenarios for a genuine allele flip. Instead of examining various scenarios in turn, we instead identify the common underlying parameters that must be affected in order to trigger an allele flip. We show that unless the sign of the mean of the distribution of the association test statistic varies between studies, the probability of observing an allele flip at a genuine causal locus in samples ascertained similarly from a common population is negligible. When the sign of the mean is reversed between studies, the probability of an allele flip increases directly with the power of the studies. We derive expressions for the mean of the odds ratio test statistic under common models that illustrate clearly how the behaviour of key model parameters impacts the probability of a genuine allele flip. In particular we show how genuine allele flips can occur even under constant LD and in regions of zero LD. Using HapMap data, and r rather than r2 to highlight previously unobserved effects, we show that well-powered studies able to detect significant associations in regions of low LD can exhibit genuine allele flips. We conclude that consideration of local LD is a critical tool for providing evidence in support of a genuine allele flip.

FOXG1 is responsible for the congenital variant of Rett syndrome. *F. Ariani[1], G. Hayek[2], D. Rondinella[1], R. Artuso[1], M. A. Mencarelli[1], A. Rosseto[1], M. Pollazzon[1], S. Buoni[2], O. Spiga[3], S. Ricciardi[4], I. Meloni[1], I. Longo[1], K. Krumina[5], M. Zappella[2], V. Broccoli[4], F. Mari[1], A. Renieri[1]* 1) Medical Genetics, Univ of Siena, Italy; 2) Child Neuropsychiatry, AOUS, Siena, Italy; 3) Biochemistry and Molecular Biol, Univ of Siena, Italy; 4) S. Raffaele Scientific Institute, Dibit, Milano, Italy; 5) Medical Genetics, Children's Univ Hospital, Riga, Latvia.

In the classic form of Rett syndrome (RTT), females are heterozygous for mutations in the X-linked MECP2 gene and the few reported males have an XXY karyotype or MECP2 mutations in a mosaic state. A number of RTT variants have been described including the congenital variant, firstly reported by Rolando in 1985. In this form, girls are floppy and retarded from the very first months of life. Using array-CGH, we identified a de novo 3 Mb deletion of chromosome 14q12 in a 7 year-old girl with dysmorphic features and a RTT-like clinical course. The deleted region contained only 5 genes. Among them, FOXG1 was very interesting since it encodes a brain specific transcriptional repressor. We analyzed this gene using both DHPLC and qPCR in 63 MECP2/CDKL5 mutation-negative RTT patients. FOXG1 point mutations were identified in 3 congenital variant patients. Two were de novo truncating mutations (p.W255X and p.S323fsX325) and one was a missense mutation (p.N227K) affecting the forkhead domain. FOXG1 encodes a protein that is highly expressed and plays an essential role during early embryonic development of the telencephalon. We found that FoxG1 expression is also detectable in the differentiating cortical compartment during post-natal stages with a profile overlapping with that of MeCP2. At single cell level, we showed that both proteins have a large co-localization domain in the nucleus but FoxG1 is excluded from the MeCP2 positive heterochromatic foci suggesting that it is not a transcriptional repressor stably associated with heterochromatin. These results suggest that FoxG1 might share common molecular mechanisms with MeCP2 during neuronal development, exhibiting partially overlapping expression domain in post-natal cortex and subnuclear localization.

The hunt for de novo chromosomal aberrations in patients with MR/MCA. *K. Kok, G. B. van der Vries, A. de Boer, Y. Swart, H. Alkema, N. Halsema, H. Zorgdrager, T. Dijkhuizen, C. M. A. van Ravenswaaij-Arts, B. Sikkema-Raddatz*
Genetics, UMCG, University of Groningen, Groningen, Netherlands.

Array-based comparative genomic hybridization has become an indispensable tool in the analyses of genome integrity and thus in the hunt for small de novo chromosomal aberrations that are presumed to be present in patients with multiple congenital abnormalities and/or idiopathic mental retardation. With the increasing resolution of this technique it was expected that the discovery rate of causally related aberrations would equally increase. The discovery of neutral copy number variants has complicated these analyses, especially since it has become clear that these variants constitute well over 5 % of the genome. There is thus a need for procedures that efficiently distinguish inherited from de novo aberrations. We have recently implemented an oligo-based array platform for the postnatal screening of patients with MR/MCA for cryptic microdeletions and duplications. Validation studies have resulted in a standard operating procedure for the routine analysis of patients using a custom designed Agilent 2x105K array in addition to karyotyping. Every patient is analysed simultaneously with his or her parents. On one array of the slide, the patient is hybridized to a reference sample constituted of a pool of either 40 males or females. On the second array, the parents are hybridized with opposite dyes. Separate 2logR files for the parents are subsequently generated by home made software of which the export files can be uploaded into several commercial data analysis platforms. In this procedure ~89% of all aberrations detected by the patient could directly be traced back to either of the parents. This approach thus constitutes a cost-efficient and fast way to determine the de novo nature of the aberrations that are seen in the patient. Detailed results on the analysis of 50 trios will be presented.

Impact of the Winner's Curse on Gene-Environment Association Studies. *M. Zawistowski, S. Zöllner* Department of Biostatistics, The University of Michigan, Ann Arbor, MI.

The challenge of replicating initial genetic associations is partly due to the Winners Curse, the systematic overestimation of effect parameters. As researchers begin to incorporate environmental exposure data into association studies it is important to understand how the Winners Curse will impact parameter estimation and false positive rates in studies of gene-environment interaction.

To explore this question we simulate case-control datasets under various models of gene-environment interaction and test for genetic association. We consider two alternative approaches for assessing association: (1) a standard Chi-Square Test (CST) for allelic association that ignores environmental status and (2) a likelihood ratio test (LRT) for marginal genotype association and/or genotype-environment interaction in a logistic regression that controls for environment. If a simulated marker is significantly associated with the phenotype ($p<10^{-6}$), we estimate its penetrance effect parameters assuming a model of additive genetic and environmental main effects and a non-additive gene-environment interaction term. Thus, we obtain the sampling distribution of genetic and interaction effects for genome scans that generated significant association in the initial test.

In the presence of main and interaction effects, both testing strategies lead to similar overestimates of effect with bias increasing as power of the initial test decreases. More surprising is the potential for the Winners Curse to induce false positives. If there are main genetic and environment effects but no interaction, subsequent hypothesis tests of interaction based on data that had a significant test result have elevated Type I Error rates. The CST introduces a slight overestimation of the interaction term but inflates the Type I Error rate by as much as 50%. The LRT results in unbiased but highly variable estimates that lead to a false positive rate nearly double what is expected. Through our simulations, we show the Winners Curse leads not only to biased estimates but also to increased false positives in gene-environment studies.

Absence of weight gain association with the *HTR2C* -759C/T polymorphism in patients with schizophrenia treated with iloperidone. *A. Thompson, C. Lavedan, S. Volpi* Vanda Pharmaceuticals Inc., Rockville, MD.

Most antipsychotics are associated with adverse effects on metabolic parameters, such as weight gain, hyperglycemia, and dyslipidemia. The identification of genetic markers associated with antipsychotic-induced weight gain may provide a powerful tool to predict individual patient response and may ultimately lead to a reduced risk for this unwanted effect. Iloperidone, a novel mixed D2/5-HT2 antagonist, has demonstrated in multiple clinical trials a low or neutral effect on metabolic parameters, such as serum levels of glucose, cholesterol, and triglycerides, and a modest short-term weight gain. Several genes have been studied for their potential role in drug-induced weight gain, including the gene for the serotonin receptor 2C *(HTR2C),* for which several antipsychotics have binding affinity. Among antipsychotics, clozapine and olanzapine are known to induce the most clinically significant weight increase, and weight gain with these drugs has been reported to be associated with the C allele of the *HTR2C* -759C/T polymorphism. In an ongoing effort to identify patients who could benefit most from iloperidone treatment, a pharmacogenetic analysis of weight changes and the *HTR2C* -759C/T polymorphism was conducted in a phase III clinical trial that included a 28-day double-blind placebo-controlled period followed by a 6-month open-label extension of iloperidone treatment. The *HTR2C* -759C/T genotype was obtained for 392 patients. A general linear model analysis of variance was performed on the change in weight from baseline up to day 203. In this study, we observed no association between weight change and the -759C/T polymorphism. Our results suggest that either this polymorphism does not play a significant role in response to iloperidone, or that the weight change observed with iloperidone is too modest to detect such an effect. Alternatively, different alterations of *HTR2C* or other unknown factors, genetic or environmental in nature, may be responsible for the weight changes observed in our study.

Amplitude-integrated electroencephalography (aEEG): a bedside monitoring tool to assess encephalopathy and seizures in patients with metabolic disorders. *C. Theda[1], C. Aygün[2], M. Toet[3], R. Hunt[4], M. Olischar[4], D. Azzopardi[5], M. DiFazio[6], A. Hamosh[1], L. DeVries[3], L. Hellstrom-Westas[7], E. Shany[8]* 1) Johns Hopkins University, Baltimore USA; 2) Ondokuz Mayis University, Samsun Turkey; 3) Wilhelmina Children's Hospital, Utrecht The Netherlands; 4) Royal Children's Hospital, Melbourne Australia; 5) Imperial College, London UK; 6) Uniformed Services University of the Health Sciences, Bethesda USA; 7) Uppsala University, Uppsala Sweden; 8) Ben-Gurion University of the Negev, Beer-Sheva Israel.

Amplitude-integrated electroencephalography (aEEG) is increasingly used, mainly in the neonatal intensive care setting, for monitoring of patients with encephalopathy and/or seizures. A limited number of electrodes are needed; the signal obtained is processed and displayed as a time compressed tracing. Pattern recognition is used to assess tracings. aEEG patterns for different degrees of encephalopathy have been defined. Seizures are identified through the combination of aEEG pattern recognition and review of the raw EEG signal. We report aEEG findings in 22 patients (pts) with metabolic disorders, including 4 pts with glycine encephalopathy (GE; non-ketotic hyperglycinemia), 4 pts with hyperammonemia (HA), 8 pts with disorders of energy metabolism (DEM), 4 pts with peroxisomal disorders and two pts with disorders of amino acid metabolism (DAAM). aEEG patterns consistent with encephalopathy (discontinuous tracing, burst suppression pattern) were present in 12 of 22 pts - most severe in pts with GE, HA and certain DEM. Seizures were detected in 14 of 22 pts: seizures accompanied encephalopathy in pts with HA, DEM and DAAM. Seizures without encephalopathy were seen in peroxisomal disorders - possibly related to the neuronal migration defects seen in these conditions. We feel that aEEG is a valuable tool in the assessment of patients with metabolic disorders manifesting with encephalopathy and/or seizures and could potentially be used to guide treatment regimens available. Efforts to develop monitoring protocols for aEEG use and data collection in metabolic patients and a web-based repository for case submission are under way (contact first author: ctheda@jhmi.edu).

Increasing the power of genetic association studies by family-based enrichment. *A. L. Maes*[1, 2, 3], *S. R. Diehl*[2, 3] 1) Early Development Statistics, Merck & Co, Inc, Rahway, NJ; 2) Center for Pharmacogenomics and Complex Disease Research, New Jersey Dental School, University of Medicine and Dentistry of New Jersey, Newark, NJ; 3) Department of Health Informatics, School of Health Related Professions, University of Medicine and Dentistry of New Jersey, Newark, NJ.

Genetic association studies of complex disease require very large sample sizes. Now that SNP genotyping costs have been greatly reduced, a studys power is often determined by number and kind of cases and controls that can be recruited. Here, we demonstrate very substantial benefits of studying cases and controls with or without, respectively, a family history of the disease. Through simulation, we compared the statistical power of various ascertainment strategies: traditional cases/controls, family history enrichment of unrelated cases/controls, reference controls and nuclear families. A disease gene along with SNPs in high, moderate and low LD to the disease gene were simulated following multiplicative, dominant and recessive models with prevalences ranging from 1% to 25%. Data sets of equal sample size were analyzed with the LAMP test of association and an Armitage trend test adjusted to account for families. For all models examined, designs with genetic enrichment of both cases and controls had the most power. In circumstances where unrelated cases with genetic enrichment did not provide a clear advantage over traditional cases (disease with highest prevalence), studying affected sibling cases did so. Choosing controls with a negative family history of disease was especially advantageous for diseases with prevalence > 10%. Nuclear families with 2 affected siblings performed better than traditional unrelated cases and controls. However, family-based designs generally were not as powerful as case-control designs with family history enrichment. Pairing genetically enriched cases with reference controls provided greater statistical power than traditional cases/controls at a 50% savings in genotyping costs. We conclude that genetic enrichment by family history is a statistically powerful ascertainment strategy for genetic association studies.

Absence of BRCA1 mutations in Familial Pancreatic Cancer Patients. *J. E. Axilbund[1], P. Argani[1,2], M. Kamiyama[2], E. Palmisano[2], M. Raben[1], M. Borges[2], K. A. Brune[2], M. G. Goggins[1,2,3], R. H. Hruban[1,2], A. P. Klein[1,2,4]* 1) Department of Oncology, Johns Hopkins University, Baltimore, MD; 2) The Sol Goldman Pancreatic Cancer Research Center, Department of Pathology, Johns Hopkins University, Baltimore, MD; 3) Department of Medicine, Johns Hopkins University, Baltimore, MD; 4) Department of Epidemiology, Bloomberg School of Public Health, Baltimore MD.

Purpose: Recent studies have suggested that mutations in the BRCA1 gene may confer an increased risk of pancreatic cancer. To determine if BRCA1 mutations explain a significant proportion of familial pancreatic cancer, we sequenced the BRCA1 gene in a large series of well-characterized patients with familial pancreatic cancer and we evaluated the pathology of breast neoplasms that developed in relatives of pancreatic cancer patients.

Patients and Methods: The BRCA1 gene was fully sequenced in 66 pancreatic cancer patients who had at least two additional relatives with pancreatic cancer from families enrolled in the National Familial Pancreas Tumor Registry. Estrogen receptor (ER), progesterone receptor (PR), HER-2 and cytokeratin (CK) 5/6 expression were evaluated by immunolabeling of 23 invasive breast carcinomas that developed in the relatives of pancreatic cancer patients.

Results: None of the 66 (0/66: 97.5% one-side CI 0-0.054%) familial pancreatic cancer patients were found to have a deleterious mutation in the BRCA1 gene. While patients were not selected based upon their family history of breast and ovarian cancer, over half of the patients whose samples were sequenced reported a family history of breast and/or ovarian cancer. One triple-negative, CK 5/6 positive breast carcinoma was observed among the 23 carcinomas evaluated.

Conclusions: Our findings suggest that mutations in the BRCA1 gene are not highly, or even moderately, prevalent in families with a clustering of pancreatic cancer, including pancreatic cancer families who report a family history of breast and/or ovarian cancer.

Detection of Low-Level Mosaicism and Placental Mosaicism Using Oligonucleotide Array CGH. *S. Scott, N. Cohen, T. Brandt, L. Edelmann* Department of Genetics and Genomic Sciences, Mount Sinai School of Medicine, New York, NY.

Microarray-based comparative genomic hybridization (array CGH) interrogates the genome for gains and losses of material with significantly higher resolution than conventional cytogenetics. Our clinical laboratory validated a previously reported whole genome oligonucleotide array design using constitutional cytogenetic abnormalities, yet little was known about the ability of this custom Agilent Technologies 44K array to detect mosaic abnormalities. To address this, we tested artificial mosaic trisomy 21 and Turner syndrome samples at various percentages of mosaicism (100% - 0%) and found as low as ten percent mosaicism to be successfully detected by array CGH. Furthermore, aneuploid fibroblast cell lines and peripheral blood specimens with mosaic karyotypes that ranged from 30% to 94% and 17% to 80%, respectively, also were successfully detected by array CGH. Interestingly, when array CGH was performed on DNA of direct chorionic villi from product of conception (POC) specimens, the results were not always concordant with the karyotype results obtained from the cultured specimen. Specifically, a male POC specimen that displayed 40% mosaicism for trisomy 2 in cultured cells had an array CGH result consistent with a balanced male karyotype when performed on DNA from the direct specimen. To further investigate discrepancies between direct and cultured POC specimens, we performed array CGH on direct DNA from POC samples with abnormal cultured karyotypes. Surprisingly, three of four samples with double trisomies on cultured cell karyotypes had only a single aneuploid abnormality by direct array CGH and all harbored the more common trisomy. This suggests that the placental mosaicism detected in these samples could reflect true fetal mosaicism as multiple trisomies in the fetus would be expected to arrest development prior to implantation. Taken together, our results indicate that oligonucleotide array CGH can detect low-level mosaicism and that analysis of POC DNA from direct villi with this technique may not yield the same result as traditional karyotype analysis, especially in cases of rare abnormalities, such as double aneuploidies and uncommon trisomies.

Association of Paternal DNA Copy Number Variation with Risk of Sporadic Retinoblastoma in an Offspring. *E. C. Chao[1], K. Wang[1], S. Walther[1], J. A. Richards[1], G. Bunin[2], A. Ganguly[1]* 1) Dept Genetics, Univ Pennsylvania, Philadelphia, PA; 2) Children's Hospital of Philadelphia, Philadelphia, PA.

Retinoblastoma(RB) is a malignant neoplasm of the retina, which occurs in infants and young children. This tumor is universally associated with mutation of the RB1 gene. In sporadic, bilateral RB, 94% of germline RB1 mutations occur prior to conception on the paternal gamete[Zhu et al., Nature 1989]. We hypothesize that paternal genotype contributes to risk of fathering a child with a de novo RB1 germline mutation. Multiple studies have identified DNA sequence and copy number variants (CNV) associated with risk of common diseases, including cancer [Wellcome Trust, Nature 2007, Xu et al., Nat Gen, 2008]. To study association of CNVs with RB risk, we analyzed genomic DNA from 69 fathers of children with de novo, bilateral RB. We used an array-based approach (Affymetrix, SNP5) to genotype >1 million loci. We applied PennCNV [Wang et al., Gen Res, 2007], a hidden Markov model based algorithm, to detect genome-wide CNVs from SNP genotyping data. In this cohort we identified 37 copy number variants >500kb, including a 1.4Mb duplication of 15q11.2 (18671827-20088816bp) in 9% of fathers.

Previous studies have linked environmental and genetic exposures to specific types of DNA mutations. We classified our subjects based on the type of mutation in their gamete as point mutations or frameshifts (small insertions/deletions). Fathers of frameshift mutations were significantly more likely (14/38 vs 3/31) to harbor a 50kb deletion within 12p13.31, downstream of the KLRB1 gene (p=0.012). This deletion contains a highly conserved noncoding element, retained over 400 million years of evolution, which suggests a regulatory role. In conclusion, heritable copy number variation may be associated with increased risk of de novo RB1 mutation on the paternal allele, and specific copy number variants may contribute to risk of different types of DNA mutations. While these preliminary results require further validation, they nevertheless suggest a novel mechanism for cancer predisposition and have broader implication for other types of de novo cancer causing mutations.

Worldwide Population Structure using SNP Microarray Genotyping. *D. J. Witherspoon, J. Xing, W. S. Watkins, Y. Zhang, L. B. Jorde* Dept Human Genetics, Univ Utah, Salt Lake City, UT.

We genotyped 348 individuals sampled from 24 populations world-wide using the Affymetrix 250k NspI microarray chip. For context, we added matching genotypes from 210 HapMap individuals for a total of 250,823 loci genotyped in 543 individuals from 28 populations. We included populations from India and Daghestan to provide detail between the genetic poles of Western Europe, East Asia, and sub-Sahara Africa. With so many markers, principal components analyses reveal genetic differentiation between almost all identified populations in our sample. Northern and southern European populations (FST = 0.004, $p<0.01$) are statistically distinguishable, as are upper and lower caste groups in India (FST = 0.005, $p<0.01$). All individuals are accurately classified into continental groups, and even between closely-related populations, genetic- and self-classifications conflict for only a minority of individuals (e.g. ~2% between upper and lower Indian castes; k-means clustering.) As expected, the HapMap CHB+JPT, CEU, and YRI samples are most similar to our east Asian, west European, and African samples, respectively. The HapMap CEU samples and our northern European ancestry samples were both collected from Utah. Although individual samples cannot be reliably classified into their collection of origin, the groups are statistically distinguishable despite their high similarity (FST = 0.0005, n.s.). Our Japanese group is also statistically distinguishable from the HapMap JPT group (FST = 0.006, $p<0.01$), and in this comparison, most samples can be correctly classified. With such large numbers of genotypes, significant differences can be found even between very similar population samplings. Our results provide guidelines for researchers in selecting suitable control populations for case-control studies.

TARDBP gene mutations in a large cohort of Italian patients with Amyotrophic Lateral Sclerosis (ALS). *L. Corrado[1], A. Ratti[2], C. Gellera[3], E. Buratti[4], B. Castellotti[3], N. Ticozzi[2], L. Mazzini[5], L. Testa[5], Y. Carlomagno[1], F. Taroni[3], F. E. Baralle[4], V. Silani[2], S. D'Alfonso[1]* 1) Dept. Medical Sciences, A. Avogadro University, Novara, Italy; 2) Dept. Neurological Sciences, IRCCS Istituto Auxologico Italiano, University of Milan, Milano, Italy; 3) U.O. Biochimica e Genetica Fondazione IRCCS - Istituto Neurologico Carlo Besta, Milano, Italy; 4) ICGEB, AREA Science Park , Trieste, Italy; 5) Dept. Neurology, A. Avogadro University and Maggiore della Carità Hospital, Novara, Italy.

ALS is a progressive and fatal adult-onset disorder characterised by the degeneration of motor neurons in the brain and spinal cord. About 5-10% are familial cases (FALS). Mutations in SOD1 are detected in 20% of FALS and 3% of sporadic (SALS) cases. However, in the majority of FALS and SALS cases the disease cause is unknown. Recent studies identified missense mutations in 1% of ALS patients in the gene (TARDBP) encoding TDP-43, the major protein of ubiquitinated inclusions in the cytoplasm of spinal motor neurons, a ALS pathological hallmark. The aim of this study was to further define the spectrum of TARDBP mutations in a large Italian cohort of ALS patients and to study the TDP-43 integrity in patients non neuronal tissue. We screened 690 ALS patients (134 FALS and 556 SALS) for TARDBP mutations. We sequenced the entire coding region for 281 patients and only exon 6 (mainly affected by mutations in the previous studies) for the remaining 409 cases. We identified 12 different heterozygous missense mutations in exon 6 (A382T in 7 patients, G287S, M337V and 9 newly identified variations) in 18 patients (6 FALS) and 0/350 healthy matched controls. The frequency of TARDBP mutations seems particularly high in Italian ALS patients (2.6% vs 1% in patients mainly of Northern European origin). Western Blot analysis of lymphocyte preparations showed aberrant TDP-43 bands (30-32 kDa) in 2 patients carrying TARDBP mutations not detected in 7 patients negative for TARDBP mutations and 4 healthy controls indicating that TARDBP mutations may affect the stability of this protein even in non neuronal tissues and that blood cells could be used to design ALS diagnostic/prognostic tools.

Views of U.S. Geneticists and Mothers of Newborns Differ Widely Regarding the Use of Residual Newborn Screening Blood Samples. *M. Lewis[1], M. Diener-West[2], G. Cutting[3]* 1) Humanities, Penn State Hershey Med Ctr, Hummelstown, PA; 2) Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; 3) Johns Hopkins University School of Medicine, Baltimore, MD.

Background: The potential research use of residual newborn screening blood samples raises many ethical questions. Yet, little is known about how mothers of newborns view these issues. Geneticists are more likely than mothers to be involved in the policy process regarding the use of these samples. Purpose: The purpose of this study is to evaluate the views of U.S. geneticists and mothers of newborns regarding these issues. Methods: The two groups were administered identical surveys in which they were asked to assume that they had just had a baby and the state could retain their babys residual newborn screening blood sample. The survey was administered orally in-person prior to discharge from the hospital to 144 mothers (response rate 95%)in Arlington, Virginia. Members of the American Society of Human Genetics with an M.D. degree, a Ph.D., or both (n=2609) were contacted by e-mail; 344 respondents completed the survey online (response rate 13%). Results: Geneticists were seven times more likely than mothers to respond that it is somewhat or completely unacceptable to seek parental permission to use residual newborn screening blood samples for research (OR= 7.1; 95% CI 1.7-29.9). Geneticists were seven times more likely than mothers to respond that permission should be obtained only once to use residual newborn screening blood samples for research rather than obtain parental permission every time researchers want to use the residual blood sample (OR= 7.3; 95% CI 4.4-12.1). Geneticists were fourteen times more likely than mothers to respond that researchers should not be required to recontact parents if they find out something that might be important to the babys health (OR= 14.1; 95% CI 4.4 -45.7). Conclusion: Views of geneticists and mothers differ significantly. These findings indicate that the public must be adequately represented in the development of policies governing the use of residual newborn screening blood samples for population-based genetic research.

A novel method to detect runs of homozygosity. *L. Licamele, N. Wang, C. Lavedan* Vanda Pharmaceuticals, Inc, Rockville, MD.

Recently there has been a large increase in the use of microarrays for whole genome association studies in search of susceptibility genes for various traits. This has led to a vast amount of data being generated but has largely failed to result in reproducible candidate genes. Lencz et al. proposed using runs of homozygosity (ROHs) to detect areas of association using microarray data because of their potential in identifying recessive traits. ROHs identify regions where individuals have inherited identical material from both parents. It is possible that some diseases may be caused by the lack of variation across one or multiple genes. We developed a precise ROH method and applied it to the study of schizophrenia. Schizophrenia is a severe psychotic disorder affecting approximately 1% of the population worldwide. The disease carries a high rate of mortality, with approximately 10% of patients committing suicide. Identification of schizophrenia susceptibility genes has remained challenging, even though genetic epidemiology studies have revealed high heritability estimates (70-80%). Our Exact ROH (eROH) method can efficiently detect ROHs in large datasets, eg, hundreds of individuals across hundreds of thousands of markers. eROH comprises three steps. 1) ROHs are selected in each individual sample according to the minimum ROH length, eg, 100 single nucleotide polymorphisms (SNPs). 2) These data are then stored into a compressed matrix containing ROH information for each SNP across all individuals. 3) Statistics are calculated across this table to dynamically find the longest ROHs that meet individual coverage criteria, eg, present in 10% of the population. Previous methods have counted SNPs present in ROHs across individuals, with no guarantee that the full ROH is in fact shared between the individuals. We evaluated eROH on a set of 426 schizophrenia samples and compared the results of the two methods. eROH is able to dynamically detect the largest ROHs in sizeable datasets while meeting the defined requirements of ROH length and individual coverage. We believe this method will allow researchers to perform informative ROH analysis while not requiring the computational power of a naive approach of evaluating every possible alignment.

Epistasis-list.org: A Curated Database of Gene-Gene and Gene-Environment Interactions in Human Epidemiology. *A. A. Motsinger-Reif[1], S. J. Wood[1], S. Oberoi[1], D. M. Reif[2]* 1) Bioinformatics Research Center, Department of Statistics, North Carolina State University, Raleigh, NC; 2) National Center for Computational Toxicology, U.S. Environmental Protection Agency, Research Triangle Park, NC.

The field of human genetics has experienced a paradigm shift in that common diseases are now thought to be due to the complex interactions among numerous genetic and environmental factors. This paradigm shift has prompted the development of myriad novel methods to detect such interactions in epidemiological studies. The success of these new methods, as well as the appropriate application of traditional approaches, in detecting such interactions is evidenced by the increasing number of epistatic models found in studies of human genetics. We have manually curated a list of such interactions from PubMed literature references, and have developed a searchable web-based tool compiling these results. This curated list is available at www.epistasis-list.org and will be updated regularly. The website is organized into a searchable list of all interactions found, as well as a searchable list of analytical tools available to detect such interactions. The organized list of interactions is searchable by disease, genetic or environmental factors detected, study design used, analytical method used, etc. The list of analytical methods is searchable by pertinent details such as study design or outcome and risk factor variable characteristics. Through the end of 2007, over 450 gene-gene and/or gene-environment interactions have been found. Breakdowns according to analytical methods and study designs used indicate some important trends. For example, ~63% of all interactions were discovered with traditional statistical methods, while ~37% used novel techniques; over 96% of studies used an association mapping approach, with only 10% of those using family-based data. It is our hope that this curated database will assist investigators in evaluation of epistasis in their own work by highlighting successful applications of appropriate experimental design and analysis. Although this work was reviewed by EPA and approved for publication, it may not necessarily reflect official Agency policy.

Analyses and visualisation of sequence data from Roche GS FLX, Illumina Genome Analyzer and ABI 3730XL.

K. A. Stangier[1]*, V. Tischler*[2] 1) GATC Biotech AG, Konstanz, Germany; 2) Fachhochschule Gelsenkirchen, Fb Angewandte Naturwissenschaften, August-Schmidt-Ring 10, 45665 Recklinghausen, Germany.

The technical capability of the Next-Gen sequencing systems, particularly embodied by the Roche GS FLX and the Illumina Genome Analyzer, generate huge amounts of sequence information. The profound bioinformatic analysis and the focused visualisation of this data is the crucial point for an in-depth comprehension of its biological relevance. GATC presents data of genomes that have been sequenced using single and paired end sequencing methods on the different systems. We recommend to use paired end reads for resequencing in order to find SNPs and other differences like InDels. With these read pairs, especially from libraries of large inserts, structural variations can be easily identified. We use a wide range of bioinformatic solutions for genome assembly, transcriptome analysis and other studies. Proprietary tools are applied for the analysis and handling of next-gen sequencing data, while third party tools are available for optimised de novo assembly of sequence data from the Genome Analyzer or improved hybrid assemblies. The GATC Genome Browser desktop application for data visualisation allows a convenient overview of e.g. whole genome de novo or re-sequencing, ChIP or SAGE experiments. The visualisation of the coverage helps to identify smaller InDels and rearrangements within the genome. In addition SNPs, coding regions and other annotations can be displayed. Conclusion: There are two main parameters which need to be observed to ensure the in-depth understanding and interpretation of the sequence data: a) the use of various next-gen systems to take advantage of each technology and b) the combination of different bioinformatic tools and their stepwise application.

The HGNC Database: an essential resource for the human genome. *M. W. WRIGHT, S. M. GORDON, M. J. LUSH, R. L. SEAL, E. A. BRUFORD* HUGO Gene Nomenclature Committee (HGNC), EMBL-EBI, Hinxton, United Kingdom.

The HUGO Gene Nomenclature Committee (HGNC) is an essential component of human genome management, and since 1989 has been the single authority for providing unique and user-friendly names and symbols for every gene in the human genome. Of the 25,000+ genes in our database most are protein-coding; we also name pseudogenes, phenotypic loci, some genomic features, and to date have named over 1,000 human non-coding RNA genes. Approved gene symbols are based on names describing structure, function or homology wherever possible. Researchers are encouraged to contact the HGNC to request or confirm the approved nomenclature for specific genes and gene groupings, or to comment on the current gene names. A panel of over 100 specialists advise on the nomenclature of specific gene families, and we consult with our International Advisory Committee on policy issues. Coordination with nomenclature committees for other species has proven invaluable; the HGNC has a very strong and active collaboration with the Mouse Genomic Nomenclature Committee (MGNC), which has proven essential in the parallel naming of orthologous human and mouse genes. In order to identify genes for orthologous naming we have developed our HGNC Comparison of Orthology Predictions search tool, HCOP (www.genenames.org/hcop) as a useful one-stop resource to summarise, compare and access various sources of orthology data. When sources agree on 1:1 orthologs between human and other mammals then these orthologs could directly adopt the human gene nomenclature. The HGNC also has a strong working relationship with other databases including Entrez Gene, Ensembl, SwissProt/UniProt and Vega. All of these databases, and many more, prominently display HGNC gene symbols; using these symbols in an online search will then allow the user to retrieve information about the genes, including the structure and function of the encoded proteins, known genetic variation and clinical phenotypes, and related genes both in humans and in other species. For further information please visit www.genenames.org or email hgnc@genenames.org. The HGNC is supported by the NHGRI & the Wellcome Trust.

Genetic profile for five common variants associated with age-related macular degeneration in densely affected families: a novel analytical approach. *L. Sobrin[1,2], J. B. Maller[2,3], B. M. Neale[2,3,4], R. C. Reynolds[5], J. A. Fagerness[2], M. J. Daly[2,3], J. M. Seddon[5]* 1) Department of Ophthalmology, Harvard Medical School, Boston, MA; 2) Center for Human Genetic Research, Massachusetts General Hospital, Boston, MA; 3) Program in Medical and Population Genetics, Broad Institute, Cambridge, MA; 4) Social, Genetic and Developmental Psychiatry Centre, King's College, London, UK; 5) Ophthalmic Epidemiology and Genetics Service, Tufts-New England Medical Center, Boston, MA.

Approximately half of the genetic variance of age-related macular degeneration (AMD) is explained by common variation at five single nucleotide polymorphisms (SNPs). We evaluated the degree to which these variants explain the clustering of AMD in densely affected families. In order to determine if the actual number of risk alleles in affected families matched the expected number, we used a novel analytical technique to simulate a comparison set of families and determine if their genetic profile differed from the observed profile in families with AMD. We genotyped 1265 siblings from 322 families with probands with advanced AMD. We used the statistical package R to create a comparison population of families. We simulated phenotypes based on the heritability and environmental variance of AMD, the allele frequencies of the five SNPs, and a liability threshold model of disease. A mean genotypic load was determined separately for simulated and actual families that shared the same configuration, i.e. the same number of affected siblings and total siblings. For most family configurations, there was no statistically significant difference between the mean genotypic loads of the actual vs. simulated families. In addition, most of the individual families genotypic loads fell within the 95% confidence interval of their expected mean genotypic load from the simulation. However, the mean genotypic load was lower than expected by the simulation in families with 4 out of 4 and 4 out of 5 siblings affected ($p=0.05$ and $p=0.020$, respectively). Given that these densely affected families may harbor more penetrant, rarer variants, linkage analyses could uncover additional implicated genes.

The role of menin in DNA damage-dependent cell cycle checkpoints. *M. C. Kottemann, A. E. Bale* Dept of Genetics, Yale University, New Haven, CT.

MEN1, the gene responsible for the cancer predisposition syndrome multiple endocrine neoplasia type I, has been implicated in DNA repair, cell cycle control, and transcriptional regulation. It is unclear to what degree these processes are integrated into a single encompassing function in normal cellular physiology and how deficiency of the MEN1 protein, menin, contributes to cancer pathogenesis. In this study, we found that loss of *MEN1* in mouse embryonic fibroblasts caused abrogation of the G1/S and intra-S checkpoints following ionizing radiation. The cyclin-dependent kinase inhibitor, p21, failed to be upregulated in the mutant although upstream checkpoint signaling remained intact. Menin localized to the p21 promoter in a DNA damage-dependent manner. The MLL histonemethyltransferase, a positive transcriptional regulator, bound to the same region in the presence of menin but not in MEN1 -/- cells. These data indicate that menin participates in the checkpoint response in a transcriptional capacity, upregulating the DNA damage-responsive target p21.

Novel 1p36 Deletion and 1q41-44 Duplication: Further Delineation of the Distal Partial Trisomy 1q Phenotype. *J. A. Gold, R. A. Cox, T. Huang* University of California Irvine Department Pediatrics Division of Genetics and Metabolism 101 The City Drive, ZC 4482 Orange , CA 92868.

Various cytogenetic abnormalities of chromosome 1 have been reported to date. The most common of these is a distal deletion of the short arm, resulting in characteristic facial features, mental retardation, growth abnormalities, and various other phenotypic anomalies. Deletions and duplications also occur on the long arm of chromosome 1, but with less reported frequency. A relatively new phenotype comprised mainly of characteristic facial features, growth retardation, microcephaly, and long and overriding toes has been postulated to arise from the distal partial duplication of the long arm of chromosome 1. We report an infant with a novel chromosomal rearrangement resulting in a deletion of 2.9 Mb at 1p36.33-p36.32 and a duplication of approximately 25.53 Mb at 1q41-q44. He presented with intrauterine growth retardation, hydrocephalus with aqueductal stenosis, and hypotonia. Clinical evaluation revealed characteristic facial features including straight eyebrows, upslanting palpebral fissures, a flat nasal bridge, a short neck, and dysplastic ears in addition to anomalies such as small hands, bilateral single palmar creases, overlapping 1st and 5th fingers, short 5th finger and clinodactyly, cryptoorchidism, a small phallus, rocker bottom feet, and 3-4 toe syndactyly. Although some of these features are consistent with the well-described 1p36 deletion syndrome, the others may contribute to distal partial trisomy of 1q.

High-quality oligonucleotide array CGH analysis using direct amniotic fluid. *A. M. Breman, P. A. Eng, S. F. Venable, W. Bi, A. Patel, S. W. Cheung, L. D. White* Medical Genetics Laboratories, Dept of Molecular & Human Genetics, Baylor College of Medicine, Houston, TX.

The use of array CGH to detect chromosome imbalance has been a major advancement in the field of cytogenetics, and the recent transition from BAC arrays to oligonucleotide arrays has greatly improved the resolution and sensitivity of this assay. Array CGH has recently been applied to prenatal diagnosis of genomic imbalance in the clinical laboratory setting, which has the potential to produce more rapid results compared to traditional karyotype analysis when direct uncultured specimens are used. While reliable results have been obtained using both direct and cultured amniocytes, direct amniotic fluid specimens often yield smaller quantities of DNA and produce noisy results on oligonucleotide arrays. We present the results of array CGH analysis on 22 direct amniotic fluid specimens. High-quality results were obtained from 20 (91%) of these uncultured samples using a minimum of 300 ng of genomic DNA. In two cases, limited DNA was isolated and whole genome amplification (WGA) was used. The results of this study include 4 cases with chromosome abnormalities and one case with a familial copy number variation. In case 1, array CGH revealed a loss of copy number on the distal short arm of chromosome 9 and a gain of copy number on the distal long arm of chromosome 16. G-band analysis revealed a derivative chromosome 9 in this fetus. In cases 2 and 3 (monozygotic twins), a loss of copy number on the short arm of chromosome 8 at band 8p23.1, including the GATA4 gene, was observed. In case 4, array CGH detected a gain in copy number on the short arm of chromosome 20. FISH analysis showed an additional partial isochromosome 20p, reported as a supernumerary marker chromosome by the laboratory performing G-band analysis. The turn-around time for array CGH analysis of direct amniotic fluid surpasses that of standard karyotype analysis. This facilitates rapid results and short reporting time (averaging 6 days). The consistent high-quality results reported here demonstrate the reliable advantage of array CGH analysis compared to conventional karyotype analysis for copy number change.

Genetic Screening in a *Drosophila* Fanconi Anemia Model. *A. P. Clark, A. E. Bale* Genetics Department, Yale University , New Haven , CT.

Fanconi Anemia (FA) is a rare, recessive, autosomal and X-linked disorder characterized by sensitivity to cross-linking agents, congenital abnormalities, pancytopenia and cancer susceptibility. FA is a genetically heterogeneous disease with 13 known complementation groups (A, B, C, D1, D2, E, F, G, I, J, L, M and N). Of these complementation groups, there are known *Drosophila* homologs for FANCD1, FANCD2, FANCI, FANCL and FANCM. A *Drosophila* FA model using a RNAi knockdown construct targeted against dmFANCD2 was previously validated in our lab. When constitutively expressed, this RNAi construct causes pupal lethality. FANCD2 knockdown flies also exhibit sensitivity to cross-linking agents, hypermutability and S phase arrest defect, demonstrating that *Drosophila* FANCD2 functions similarly to its mammalian homolog. An EP element screen was performed to identified genes that, when misexpressed, rescue the pupal lethality phenotype associated with dmFANCD2 knockdown. Two EP element lines that partially rescue pupal lethality were identified: line 17396 and line 20140. The EP element in line 17396 sits upstream of CG30122, the *Drosophila* homolog of heterogeneous nuclear riobonucleoprotein U like 1 (hnRNPUL1) and causes hnRNPUL1 overexpression in combination with an actin Gal4 driver. The EP element in line 20140 is positioned upstream of Vha44, a component of the vacuolar H+ ATPase. Neither of these EP elements rescue the S phase defect seen in FANCD2 knockdown flies. Current efforts are focused on elucidating the function of hnRNPUL1 and Vha44 in DNA repair and apoptosis.

Shared Genetic Loci for Language Impairment and Reading Disability. *S. D. Smith[1], M. L. Rice[2]* 1) Dept Pediatrics, Univ Nebraska Medical Center, Omaha, NE; 2) Dept Speech, Language, Hearing, University of Kansas, Lawrence, KS.

Specific language impairment (SLI), reading disability (RD), and speech sound disorder (SSD) are clinically distinguishable learning disorders with a high degree of comorbidity. All three disorders have substantial heritabilities, leading to the hypothesis that they share genetic risk factors affecting a common neurodevelopmental pathway. At least 9 loci have been linked to RD, and SSD has been linked to 3 of those loci on chromosomes 3, 6, and 15, indicating that genes may exist in these regions that affect both disorders. In contrast, genome-wide linkage analyses of SLI have identified loci on chromosomes 16 and 19, with other loci possible on chromosomes 7 and 13. This suggests that SLI has a different genetic etiology and therefore follows a different developmental pathway. To test whether previous genome wide searches were underpowered to detect shared but lesser influences from the RD/SSD loci, we performed a targeted linkage analysis in families of probands with SLI. Probands and their siblings from 86 families totaling over 150 sib pairs were assessed for measures of language, reading, and articulation, and genotyping was performed for microsatellite markers at 2 cM intervals covering 5 chromosomal regions: RD candidate regions on chromosomes 1p36, 3p12-q13, 6p22, and 15q21, and the language candidate region on 7q31. Quantitative sib-pair linkage analysis was done using two different analytical methods, and replication of linkage was accepted only if both methods indicated linkage of the same phenotypes to the same markers. Linkage was replicated or suggestive in all of these regions, and phenotypic analysis indicated that the genetic influences may be domain-specific. Language phenotypes showed greater linkage with 7q, speech phenotypes showed greater linkage with chromosome 3 markers, and reading phenotypes showed greater linkage with 1p, 6p and 15q, consistent with previous findings for these regions. These results support a multiple gene model of the comorbidity between language impairments and reading disability and have implications for neurocognitive developmental models and maturational processes. Funded by NIDCD DC01803.

Analysis of atypical patients with features overlapping Pallister-Hall and Greig cephalopolysyndactyly syndrome. *J. J. Johnston, J. Sapp, J. Turner, L. G. Biesecker, Clinical Collaborators* NHGRI, NIH, Bethesda, MD.

Pallister-Hall and Greig cephalopolysyndactyly syndromes are caused by mutations in the *GLI3* transcription factor on chromosome 7p14.1. Although variant phenotypes, including isolated polydactyly, acrocallosal syndrome, and severe mental retardation, have been attributed to mutations in *GLI3*, atypical phenotypes attributable to such mutations are not adequately delineated. To better understand the clinical variability resulting from mutations in *GLI3* we have studied a cohort of patients who have features of PHS and/or GCPS but do not fulfill the clinical criteria for either disorder. The group consisted of 55 probands and 6 family members. Twenty-two probands had features of GCPS including polydactyly, syndactyly, hypertelorism, macrocephaly, and/or agenesis of the corpus callosum. Twenty-three probands had features of PHS including polydactyly, hypothalamic hamartoma, panhypopituitarism, imperforate anus, and/or hypoplastic nails. Ten probands had either hamartoma or polydactyly without additional overlapping features of either disease. We identified a total of 17 mutations including one missense variant. *GLI3* has a well-established genotype/phenotype correlation with truncating mutations in the middle third of the gene, between nt 1198-3481, causing PHS and mutations outside this region causing GCPS. Of the 6 probands with features of GCPS, 3 had truncating mutations in *GLI3* that fell outside the middle third of the gene, one had a splice site mutation upstream of the middle third of the gene, one had a missense alteration, p.S903L, and one had a mutation at nt 3474 at the PHS/GCPS boundary. Nine probands with features overlapping PHS had truncating mutations in *GLI3*, all fell in the middle third of the gene. No individuals with polydactyly or hamartoma in the absence of other overlapping features had mutations in *GLI3*. In summary, individuals with multiple features of PHS and GCPS should be considered for *GLI3* mutation screening.

Increased Attention Deficit Hyperactivity Disorder (ADHD) and Major Depression (MD) symptoms in Nail Patella Syndrome (NPS): potential association with LMX1B loss-of-function. *I. McIntosh[1,2], E. Sparrow[3], C. Slavin[2,3], J. James[3], J. E. Hoover-Fong[2], E. Tierney[2,3]* 1) American University of the Caribbean, St Maarten, Netherlands Antilles; 2) Inst of Genetic Med, Johns Hopkins Univ, Baltimore, MD; 3) Kennedy Krieger Inst, Baltimore, MD.

ADHD has a prevalence of 2-7% in USA adults, with a male to female ratio (M:F) of 4:1. MD has a prevalence in USA adults of 5% (M:F 1:1.7). NPS (OMIM 161200) is an autosomal dominant disorder that is the result of loss-of-function mutations in LMX1B, a LIM-HD transcription factor. Lmx1b has been shown to be expressed in mesencephalic dopaminergic neurons including the substantia nigra pars compacta & ventral tegmental area. We hypothesized that individuals with NPS would present with ADHD & MD symptoms (sxs) and that NPS may yield additional information about the etiology of ADHD & MD. The Conners CAARS-S:L Scale (CAARS) & Beck Depression Inventory-II (BDI), to assess for presence and severity of ADHD & MD sxs respectively, were completed by 51 adults with NPS (39 females & 12 males, age 46.313.9 yrs). The Brief Pain Inventory (BPI) was also completed by 17 of 51 (14 females & 3 males, age 52.512.7 yrs). The CAARS scores are based on ratings of the 18 DSM-IV criteria for ADHD. Of the 51 NPS subjects, the following had scores that reached the threshold of sxs consistent with ADHD: TE-Inattention 11 (22%); TF-Hyperactive-Impulsive 7 (14%); TH-ADHD Index 8 (16%). The threshold TH M:F was 1.1:1. Nineteen (37%) had BDI-Total score (BDI-T) that reached threshold of 14, consistent with depression (M:F 1:1.5). Multiple regression analysis (MRA) showed the higher the TH, the greater the BDI-T (p=.009). TE, TF, TH & BDI-T did not correlate with sex or age. MRA of BDI-T & BPI Interference was significant only for walking ability (p=.05). The rate of clinically significant ADHD sxs in NPS was 2-8 times greater than the USA prevalence and did not show a M:F bias. The rate of significant MD sxs was 7 times the USA rate and did not correlate with report of pain. Future studies should be designed to better assess cognitive abilities, mood & motor deficits, and to confirm the increased rates of ADHD & MD found with screening tests.

Adult osteogenesis imperfecta model (*oim*) mice do not exhibit inherent muscle weakness or pathology. *C. Phillips[1], B. Weber[2], S. Carleton[1], A. McCambridge[1], J. Ferreira[2], M. Brown[2]* 1) Dept Biochemistry/Child Health, Univ Missouri, Columbia, MO; 2) Dept Veterinary Pathobiology, Univ Missouri, Columbia, MO.

Bone is inherently mechanosensitive, responding and adapting to its mechanical environment. Bone formation occurs in response to high mechanical loads; often changing geometry to strengthen the skeleton. The largest physiological loads bones typically experience are from muscles; bone strength is proportional to muscle mass. Osteogenesis imperfecta (OI) is a heritable connective tissue disorder characterized by small stature, reduced bone mineral density (BMD) and frequent fractures. OI patients reportedly have muscle weakness, though it is unclear if this is due to inactivity or inherent pathology. We used *oim* (osteogenesis imperfecta model) mice with reduced bone biomechanical integrity and BMD to investigate whether *oim* mice have inherent muscle pathology. At 4 months of age (peak femoral bone mass in mice) wildtype (wt), heterozygous (*oim*/+) and homozygous (*oim/oim*) mice were weighed and the contractile tension generating capacity of the gastrocnemius (G), soleus (S), plantaris (P) and tibialis anterior (TA) muscles measured. *Oim/oim*, *oim*/+ and wt quadriceps (Q), G, S, P, and TA muscles were harvested and histologically evaluated for morphology, muscle fiber types (myofibrillar ATPase activity), and fiber cross-sectional areas. Muscle mass/body mass ratios for the G, Q, S and P muscles are similar between wt, *oim*/+ and *oim/oim* mice. Q, TA, G, P and S muscles in *oim*/+ and *oim/oim* mice do not show evidence of necrosis, degeneration, regeneration, atrophy or hypertrophy upon microscopic evaluation. Cross-sectional fiber areas of the TA, S, and Q muscles are not significantly different in *oim/oim* or *oim*/+ mice as compared to wt mice. Muscle fiber type composition in the S muscle is similar between wt, *oim*/+ and *oim/oim* mice. Contractile generating capacity [peak tetanic tension] and nerve conduction of muscles is not impaired in *oim*/+ or *oim/oim* mice as compared to wt mice. Our findings suggest that *oim* mice do not have inherent muscle pathology and should be able to undergo therapeutic inclusion of exercise strategies to improve muscle mass and ultimately bone strength.

Using random forest for multi-marker predictive pharmacogenetics study. *N. Bing, P. St. Jean, I. Grossman, M. Mosteller* Genetics, GlaxoSmithKline, Research Triangle Park, NC.

The clinical goal of a pharmacogenetics (PGx) study is to enable the prospective identification of individual variations in drug response using a genetic marker, or a set of genetic markers. Predictive models can be used to provide rules for combining a series of genetic markers that best predict the phenotype of response to treatment. Random Forest (RF) is one such predictive modeling method, which has recently attracted much attention in genetics investigations due to its ability to (1) explore high order marker interactions; (2) handle genetic heterogeneity; (3) rank markers by importance when they are being incorporated into the multivariate prediction process; and (4) exhibit excellent performance in prediction accuracy. However, there is no clear criterion for marker selection within the RF modeling process. We therefore explored the usefulness of cross-validation (CV) as a method for marker selection in RF within the context of PGx studies. We evaluated CV in RF using both simulated and real clinical PGx data, where phenotypes are defined from adverse drug events as case or control status and genotypes are examined from more than 7000 single nucleotide polymorphisms in a panel of 120 candidate genes. Within simulation, case-control status is determined by underlying causal genetic variants and their interactions; in the meanwhile, the sample size, case event rate and genotype linkage disequilibrium relations are kept intact to mimic realistic PGx data. Simulations under various genetic interaction models indicate that CV can be used to identify correct combinations of sets of markers for RF model construction; and that marker selection by CV increases RF prediction accuracy. By contrast, marker selection via either sample fitness criteria or out of bag (OBB) error results in over-fitted models and incorrect or unnecessarily large sets of markers in the RF model. Results of applying CV for marker selection in RF to real clinical PGx data will also be discussed. Overall, we recommend that CV for marker selection be included as an essential step when using RF for building multi-marker predictive models for PGx studies.

A novel algorithm and software for evaluation of population level linkage disequilibrium (LD) patterns based on r^2. *K. K. Nicodemus[1,2], W. F. Bodmer[3]* 1) Centre for Human Genetics, Wellcome Trust, University of Oxford, Oxford, United Kingdom; 2) Department of Clinical Pharmacology, University of Oxford, Oxford, United Kingdom; 3) Cancer and Immunogenetics Laboratory, Weatherall Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, United Kingdom.

Linkage disequilibrium patterns vary between populations due to genetic drift, natural selection and admixture; metrics have been derived to measure LD including D and r^2. Additional methods have been developed to describe blocks of correlated SNPs based on D e.g., HAPLOVIEW. Clusters of correlated SNPs are commonly-used in association studies for selection of tagSNPs, defining haplotypes for association analysis and fine-mapping of putative causal loci; the use of haplotypes in detection of population substructure has not been fully explored. D is known to show a ceiling effect when only 3 of the 4 possible 2-SNP haplotypes are observed and thus is less informative than r^2 in tight-LD regions.

We present a set of new algorithms for clustering correlated SNPs based on the LD metric r^2; the 1st-level clustering algorithm accepts genotype-level data or phased haplotypes as input and allows users to impute missing genotypes, set an r^2 threshold for block definitions and set the windowsize for growing blocks, removing SNPs within the window not passing the threshold. The 1st-level algorithm may be used alone for defining haplotype blocks or users may pass the results of the algorithm to a 2nd-level meta-blocking algorithm which clusters correlated haplotype blocks and/or singleton SNPs, allowing for in-depth description of LD structure and facilitating fine-mapping. In addition, the meta-blocking algorithm may be used to detect population substructure by using meta-blocks as input to e.g., principal components analyses; differences in haplotypic LD structure are more informative than that of single SNPs. The **r2blocks** and **metablocks** functions are implemented in a freely-available package for the R statistical computing environment and we showcase its functionality on data simulated under the coalescent and on genomewide data from the HGDP-CEPH Human Genome Diversity Panel in multiple worldwide populations.

Screening of 144 patients with mental retardation and /or developmental delay by array-CGH. *F. Bena[1], S. Gimelli[1], S. Dahoun[1], N. Brun[1], S. Fokstuen[1], A. Giacobino[1], B. Conrad[2], M. A. Morris[1], A. Bottani[1], S. E. Antonarakis[1]* 1) Dept Medical Genetics, Univ Medical School and Hospital, Geneva, Geneva 4, Switzerland; 2) Dept Medical Genetics, Inselspital, Berne, Switzerland.

Array-Comparative Genomic Hybridization (aCGH) allows a high resolution whole genome analysis of copy number changes and can reveal submicroscopic deletions and duplications. We present the results of aCGH analysis in 144 probands with mental retardation, developmental delay, and/or congenital malformations. An oligo aCGH with an average coverage of 40 kb (Agilent 244K) was used. De novo Copy Number Variants were identified in 11.1% of the cases including 9 deletions (15q25.3q26.1, 22q13.3,6q13q14.1, 14q32.2q32.31, 5q14.3, 12q13.11, 10q11.22q11.23, 12p11.23, 1q21.11, 5 duplications (16p13.3, 5q14.3q15, 15q11q13, 16p12.3, Xq27.3q28), 2 deletions associated with duplications (6p25p24.3 and 6p24.3p24.1, 14q32.3 and 14q32.3), and one case with more complex rearrangement, ranging from 0.007 Mb to 13.3 Mb. aCGH analysis of patientss parents was required in 20% of these cases. Additionally, the analysis revealed 16 cases patients with novel Copy Number Variants inherited from a healthy parent ranging between 30 kb and 1.2 Mb. Our cohort also includes four cases of de novo apparently balanced translocations in which aCGH did not detect cryptic genomic unbalances at the breakpoints. As positive control, five cases of unbalanced karyotypes were analysed and revealed large deletions and duplications. We conclude that aCGH substantially contributes to the diagnostic evaluation of patients, but the interpretation of the results requires the collective effort of many laboratories and large numbers of cases and controls examined.

Vitamin D pathway gene polymorphisms and risk of breast cancer in Cyprus. *A. Hadjisavvas, M. Loizidou, A. Takousi, T. Michael, K. Kyriacou* Department of EM/Molecular Pathology, The Cyprus Institute of Neurology & Genetics, Nicosia, Cyprus.

Vitamin D plays a key role in cell proliferation, differentiation, and apoptosis in both normal and malignant breast cells. Studies have hypothesized that the active form of vitamin D has anti-proliferative effects in breast cells. This hypothesis is supported by the reduced breast cancer risk observed among women with high vitamin D intake. The involvement of vitamin D pathway gene polymorphisms (SNPs) and more specifically vitamin D receptor (VDR) SNPs in breast cancer etiology has long been of interest. In the present study we explored the implication of vitamin D pathway gene polymorphisms in breast cancer in the Cypriot population. A total of 2286 DNA samples from Cypriot women (1109 breast cancer patients and 1177 age-matched healthy controls) were genotyped for 8 SNPs in genes involved in the vitamin D pathway. Four SNPs located near the VDR 3 region have been examined; these SNPs are identified by their restriction endonuclease cleavage sites (TaqI, BsmI, ApaI and FokI). In addition, a poly(A) microsatellite repeat in the 3' untranslated region which may influence VDR mRNA stability was assessed. We also genotyped common SNPs in vitamin D pathway genes and more specifically T432G (rs7041) and A436C (rs4588), both in exon 11 of the GC gene, which codes for the vitamin D-binding protein (DBP) and rs2296241 in exon 4 of the CYP24A1 gene, which encodes 24(OH)ase, which initiates degradation of 1,25(OH)2D. The prevalence of the 8 SNPs was compared between cases and controls. Genotype frequencies were compared across groups using the chi square test and the Mantel-Haenzel test for linear trend. The association between breast cancer and each SNP was examined using logistic regression with the SNP genotype tested under models of complete dominance and recessive inheritance. Statistically significant results were observed for one of the SNPs under study. We are currently expanding our analysis to study haplotypes and their association with breast cancer.

Global microRNA analysis in human skin reveals post-transcriptional regulatory networks involved in psoriasis susceptibility and pathogenesis. *C. Joyce, A. Bowcock* Dept of Genetics, Washington Univ, Saint Louis, MO.

Psoriasis is a chronic inflammatory skin disease affecting 2-3% of the European population. Hallmarks of the disease include poorly differentiated, hyperoliferative keratinocytes, and infiltration of activated immune cells into the dermis and epidermis. In addition to these histological changes, our laboratory and others have identified many mRNAs that are misexpressed in psoriasis. MicroRNAs (miRNAs) are endogenous, small RNA molecules that post-transcriptionally regulate gene expression. Recent studies have demonstrated the importance of miRNAs in skin development and the ongoing program of terminal keratinocyte differentiation. Hence, dysregulation of miRNAs may be involved in psoriasis susceptibility and pathogenesis. To investigate this hypothesis, miRNA profiling of psoriatic lesions, matched uninvolved skin, and non-diseased controls was performed by hybridization of RNAs to Exiqon microarrays. Differentially expressed miRNAs were validated by qRT-PCR. We identified five microRNAs that were differentially expressed in psoriatic lesions and, to a lesser extent, matched uninvolved skin compared to non-diseased controls: miRs -133a/b were downregulated while miRs -21, -31, and -223 were upregulated. We also confirmed altered regulation of miR-203 which has been shown to play a role in terminal differentiation of keratinocytes and to be highly upregulated in psoriasis by Sonkoly *et al.* (2007). To determine which cell types express our novel miRNAs, we performed qRT-PCR in cell lines derived from keratinocytes, T and B cells. miR-31 was primarily expressed in keratinocytes, miR-223 was primarily expressed in T cells and B cells, and miRs-21 and 133a/b were expressed in all cell lines at high and low levels, respectively. Knockdown of miR-31 and miR-203 was successfully achieved in keratinocytes using locked nucleic acid knockdown probes. Peptide targets of these miRNAs are being identified by *in silico* approaches and by global protein analyses in wild-type and knockdown strains. These findings have implications for psoriasis pathogenesis and may provide novel methods of therapeutic intervention.

Complementing Mutation-Driven Databases with Phenotype-Rich Repositories: The Clinical and Functional TRanslation of CFTR (CFTR2) Project. *P. R. Sosnay[1], C. Castellani[2], C. Penland[3], J. Zielenski[4], G. R. Cutting[1]* 1) Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD; 2) Cystic Fibrosis Centre, Ospedale Civile Maggiore, Verona, Italy; 3) Cystic Fibrosis Foundation, Bethesda, MD; 4) Hospital for Sick Children, Toronto, Canada.

Locus-specific mutation databases (LSDBs) are populated by alleles discovered in research and diagnostic laboratories but frequently contain insufficient data to interpret the phenotypic consequences of each variant. Enhancing the accuracy and depth of clinical or functional information in existing LSDBs by retrospective data collection is expensive and inefficient. Cystic fibrosis (CF) provides an example of the challenges posed by translation of genetic variation to medical practice. The CF Mutation Database contains over 1500 disease-associated CFTR mutations, but the functional and clinical consequences have only been well characterized for about thirty mutations. The Clinical and Functional TRanslation of CFTR (CFTR2 Project) proposes to annotate all reported CFTR mutation using rigorously vetted clinical data. The curators of the CF Mutation database are performing an extensive international survey of academic and commercial laboratories that scan or sequence the CFTR gene to create a comprehensive catalogue of variations in CFTR. Clinical information agreed upon by a panel of CF experts is being extracted from Patient Registries and from major CF Care Centers from around the world and entered into the CFTR2 database. CFTR mutations with inadequate or inconsistent clinical information will be evaluated in vivo for putative RNA processing defects or in cell-based systems for putative deleterious amino acid substitutions. These functional studies will be incorporated into the assessment of disease-liability and the potential for mutation specific therapeutics. The CFTR2 project presents a novel approach to clinical and functional annotation of mutations identified in disease-causing genes. Creation of separate phenotype-driven databases for single gene disorders allows preservation of established mutation-driven LSDBs while providing expert clinical assessment for medically significant alleles.

A Single Nucleotide Polymorphism (SNP) at DBH is associated with Alzheimer Disease in European-American Subjects. *Y. Tang[1], J. Lah[3], A. Rosen[3], A. Levey[3], J. F. Cubells[1,2]* 1) Dept of Human Genetics, Emory University, Atlanta, GA; 2) Dept of Psychiatry and Behavioral Sciences, Emory University, Atlanta, GA; 3) Dept of Neurology, Emory University, Atlanta, GA.

Background: Degeneration of the locus coeruleus (LC), the largest nucleus of norepinephrine (NE)-producing neurons innervating virtually all cortical and subcortical areas, is a well described yet under-studied neuro-pathological feature of Alzheimer disease (AD). Dopamine -hydroxylase (DH), encoded by the locus DBH on human chromosome 9q34, catalyzes the conversion of dopamine (DA) to NE and is expressed specifically in the LC and other NE-containing nuclei. We hypothesized that DBH sequence variation associates with AD or AD-related behavioral phenotypes. **Method:** Nine SNPs at DBH were selected based on assay availability, inter-marker distance and linkage disequilibrium (LD) relationships. Genotypes were determined using the Taqman platform in 295 samples from AD cases and 305 healthy elderly controls of self-reported European-American ancestry. Association data were analyzed in SPSS, haplotype frequencies were calculated using WHAP, and LD structure was examined using Haploview. **Results:** One SNP, rs2519148, located approximately 7 kilobases upstream of the translation-initiation codon of DBH, associated with the diagnosis of AD in EA samples (permutation p=0.002, significant at = .05 after correction for multiple testing), with allele G and genotype GG being over represented in cases. The SNP is not in LD with either of two known putatively functional SNPs, rs1611115 and rs6271. Binary logistic regression analysis showed that genotype at rs2519148 remained significantly associated with AD after controlling for age and gender (p=0.004). **Conclusions:** The results provide preliminary support for an association between sequence variation at DBH and AD diagnosis in EA subjects. **Support:** Alzheimer Disease Research Center grant P50 AG025688 to AL.

Interaction of the cilia-centrosomal protein Retinitis Pigmentosa GTPase Regulator (RPGR) with specific Bardet Biedl Syndrome (BBS) proteins implicates RPGR-BBS complex integrity in the pathogenesis of ciliopathies. *H. Khanna[1], S. He[1], N. Katsanis[2], A. Swaroop[1,3]* 1) Ophthalmology & Visual Science, University of Michigan, Ann Arbor, MI; 2) McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD; 3) Neurobiology Neurodegeneration & Repair Laboratory, National Eye Institute, MD.

The dynamic behavior of cellular processes (such as ciliary transport) is mediated by distinct protein complexes that function as part of intricate networks or pathways. Mutations in Retinitis Pigmentosa GTPase Regulator (RPGR), a ciliary protein, are associated with a majority of XLRP cases and with hearing disorders. However, the function of RPGR in cilia-mediated regulatory pathways is poorly understood. Since retinal degeneration is a typical feature in Bardet-Biedl Syndrome (BBS), a syndromic ciliopathy, we hypothesized that RPGR associates with BBS proteins in the retina and that disruption of such interactions underlies the associated retinal degeneration in XLRP as well as BBS patients. Here we show that RPGR interacts directly with BBS4 and not with BBS2, BBS5, BBS6 or BBS8. Full-length BBS4 interacts with the amino-terminal RCC1-like domain of RPGR. Co-immunoprecipitation studies using bovine retinal extracts demonstrate that RPGR exists in complex with BBS2, BBS4, BBS6, and CEP290/BBS12. We predict the stability of RPGR-BBS complex(es) is critical for normal ciliary transport cascades and that RPGR mutations may act as modifiers of the penetrance and severity of the retinal degeneration phenotype in BBS patients.

SNP-based determination of 22q11 copy number variants in a schizophrenia trial. *P. Oeth[3], J. Crowley[1], T. Shi[3], J. Olson[3], J. Sebat[2], P. Sullivan[1]* 1) Department of Genetics, University of North Carolina, Chapel Hill, NC; 2) Cold Spring Harbor Laboratory, Cold Spring Harbor, New York; 3) Research & Development, Sequenom, Inc, San Diego, CA.

Deletion of 22q11 represents the most frequent deletion syndrome in humans, occurring in approximately 1 in 4000 live births. This deletion is currently believed to be the most common genetic risk factor for psychosis, but its prevalence in schizophrenia is unclear. To address this issue, we have examined copy number status at 22q11 in 741 individuals with schizophrenia from the CATIE clinical trial along with 733 matched controls. We first analyzed probe intensity data from a genome-wide association study of these samples using a Hidden Markov Model (HMM) for CNV prediction. We identified 2 schizophrenia cases and 0 controls with deletions >1 Mb on 22q11 (confirmed by Q-PCR), yielding a deletion prevalence in cases of 0.27% (95% CI, 0-0.64%). There were also a number of smaller predicted CNVs requiring confirmation. For this process, we employed a quantitative MassARRAY SNP-based CNV detection methodology. For an initial follow-up of the region, 109 SNPs with a HapMap minor allele frequency of 0.30 or higher were selected and multiplexed with 8 non-variant control SNPs from 4 genes located outside of the 22q11 region. Forty-eight samples, consisting of 24 cases, 18 matched controls, and 6 samples with known copy numbers for this region (CNV controls) were quantitatively genotyped. The results showed 39 SNPs deviating from the 1:1 ratio of a normal heterozygote in at least 1 or more clinical samples but showing no deviation in control SNPs for the same samples. The correlation between the HMM predictions and these results were promising with confirmation in 17 out of 41 individuals, with 12 individuals exhibiting multiple confirmatory loci. Clear allele-specific CNV status was resolved for duplications in these individuals. For example, an A/G SNP in the COMT gene exhibits a clear duplication of the A allele in two schizophrenia samples. Current work is focused on converting informative assays to resolve absolute copy numbers and expand the sample set to the entire CATIE cohort.

BRACHYOLMIA WITH AMELOGENESIS IMPERFECTA: FURTHER EVIDENCE OF A SINGLE ENTITY.

D. Bertola[1], R. Antequera[2], M. J. Rodovalho[1], R. S. Honjo[1], L. M. J. Albano[1], I. M. Furquim[1], L. A. Oliveira[3], C. A. Kim[1] 1) Pediatrics, Instituto da Criança, São Paulo, Brazil; 2) Dentistry Division, University of São Paulo, São Paulo, Brazil; 3) Radiology, University of São Paulo, São Paulo, Brazil.

Brachyolmia is a heterogeneous group of skeletal dysplasia characterized by generalized platyspondyly without significant epiphyseal or metaphyseal involvement. Several types of brachyolmia were described: (1) Hobaek and Toledo types, (2) Maroteaux type, and (3) an autosomal dominant type, in contrast to the inheritance of the others, all considered autosomal recessive (Shohat et al., 1989). In 1996, Verloes et al. described a further type of brachyolmia associated with amelogenesis imperfecta in two siblings born to a consanguineous Moroccan couple. The molecular basis of these different types of brachyolmia remains unknown. We evaluated three cases, two siblings and an isolated case also from consanguineous matings. The three affected patients presented with short stature, platyspondyly, oligodontia and enamel dysplasia. In the two siblings (ages 26 and 17) the skeletal findings were more striking with scoliosis, more pronounced in the older patient, narrow intervertebral distance, short pedicles, posterior scalloping and herniation of the nuclei; short ilia and broad femoral necks. Neither metaphyseal nor epiphyseal involvements were observed. Orthopantographic study disclosed agenesis of teeth in the three affected patients. Ophthalmologic evaluation and urinary excretion of mucopolysaccharides were normal. Our two families showing involvement of the spine and teeth give further support to the fact that skeletal and dental findings are part of the spectrum of one single entity and not a co-occurrence of two separate disorders, brachyolmia and amelogenesis imperfecta. Within the group of brachyolmia, the complete revealing of the molecular mechanisms underlying the different clinical forms of brachyolmia will help elucidate if these conditions are due to genetic heterogeneity or simply variable expressivity of the same disorder.

Generation and characterization of brain-targeted lysosomal -galactosidase for expression in microencapsulation therapy for GM1 gangliosidosis. *M. D. Lambourne, M. A. Potter* Medical Sciences, McMaster University, Hamilton, ON, Canada.

GM1 gangliosidosis is a neurodegenerative lysosomal storage disorder caused by an inherited deficiency of the enzyme -galactosidase (GLB1). Reduced catalytic activity of GLB1 results in the abnormal storage of glycoconjugates such as GM1 ganglioside, a major functional component of neuronal cell membranes, and typically leads to developmental arrest and death within the first few years of life. Microencapsulation therapy may provide an effective long-term treatment for GM1 gangliosidosis, whereby recombinant cells secreting a desired protein are implanted within a biocompatible polymer, but like other attempts to treat this disorder would be futile since the blood-brain barrier (BBB) obstructs the delivery of most therapeutics to the central nervous system (CNS). A 29 amino acid peptide derived from the rabies virus glycoprotein (RVG) can mediate transcytosis of small molecules across the BBB, and thus reveals a potential mechanism to safely target the CNS via the intravascular route. To determine whether a GLB1-RVG fusion protein would retain its enzymatic properties, the human GLB1 cDNA was cloned into an expression vector and modified to encode the RVG peptide sequence, and an Au1 epitope tag to facilitate detection. The Au1GLB1 and Au1GLB1-RVG constructs expressed in GM1 fibroblasts showed a GLB1 specific enzyme activity that was not abrogated when compared to cells expressing unmodified GLB1, and was able to restore GLB1 activity to greater than normal levels in these deficient cells. Enzyme secreted into the medium from microencapsulated cells was able to cross-correct the enzyme deficiency of cultured GM1 fibroblasts in a mannose-6-phosphate (M-6-P) dependant manner, while only the RVG peptide could facilitate M-6-P independent uptake by a neuronal cell line. Correct processing and routing of the protein from the supernatant to the lysosomes was verified for RVG-fused and unfused GLB1. These results indicate that GLB1-RVG fusion protein retains its lysosomal enzymatic properties when secreted by microencapsulated cells, and will serve as a good model to elucidate the ability of RVG peptide to allow transcytosis of large molecules across the BBB.

A flexible and powerful statistical method for evaluating genetic associations in families or unrelated subjects. *S. R. Diehl*[1, 2], *A. L. Maes*[1, 2, 3], *F. Kuo*[1] 1) Center for Pharmacogenomics and Complex Disease Research, New Jersey Dental School, University of Medicine and Dentistry of NJ, Newark, NJ; 2) Dept of Health Informatics, SHRP, University of Medicine and Dentistry of NJ, Newark, NJ; 3) Early Development Statistics, Merck & Co. Inc., Rahway, NJ.

Whole-genome and candidate-gene association studies of complex disease require very large numbers of subjects. Careful consideration of alternative study designs and the statistical tests is needed to maximize chances of success. As genotyping costs have decreased immensely, a studys power now often depends primarily on the cases and controls available. Investigators often have a mixture of unrelated subjects as well as small and larger families with one or more members affected by the disease of interest. Here, we present a flexible statistical method that accommodates both families and unrelated subjects, or combinations of both, and that provides power equal to or greater than existing methods. Using simulation, we compared the statistical power of our adjusted Armitage trend test (ATT) that accounts for family relatedness to existing family-based tests of association. We employed permutation-based adjustments to the Armitage ^{2} so the test could be applied to family data with unbiased results. Power was compared to that obtained using FBAT, PDT, and LAMP tests of association. Various nuclear family pedigree structures were studied. A disease gene along with SNPs in high, moderate and low LD to the disease gene were simulated following multiplicative, dominant and recessive models with prevalence ranging from 1% to 25%. For all pedigree structures and disease models we examined, the ATT was valid for family data (had nominal Type I error), and it very substantially outperformed both the FBAT and PDT in statistical power. The LAMP test had slightly higher statistical power than the ATT. However, the LAMP is limited to simple nuclear families while the ATT can accommodate large and complex pedigree structures with missing data. A computer program for implementing the test is available. The ATT is a powerful and flexible test of genetic association suitable for case-control and family data.

Sequence variants in the 3UTR of MECP2 gene and their potential effects on putative MicroRNA target sites. *Y. Yang, P. Fang, P. Ward* Dept Molec & Human Gen, Baylor Col Med, Houston, TX.

MicroRNAs (miRNA) are small non-coding RNAs of 18-24nt which can repress post-transcriptional gene expressions by binding to the 3UTR of targeted genes. Deregulations of miRNAs are associated with human diseases such as cancer and heart disease, and sequence variations in miRNA target sites in 3UTR can affect phenotypes. *In Silico* predictions showed that many genes, including the gene of our interest, MECP2, are abundant in putative miRNA target sites in their 3UTR regions. Sequence changes in 3 UTR of the MECP2 gene have been reported but their effects are mostly unknown. Those variants, if in the right locations, may potentially strengthen or weaken miRNA target sites, leading to increased or decreased MECP2 expression levels respectively and resulting in disease.

Our laboratory offers clinical tests for MECP2 gene sequencing, which covers the entire coding region of the MECP2 as well as a small portion of the 3UTR sequence. Seven sequence variants have been detected in the 3 UTR of the MECP2 gene by our routine sequencing analysis. While three out of the seven 3UTR changes are polymorphisms, the other four changes are unclassified variants. Using the TargetScan program, two unclassified variants, *13CT and *14GA, are ruled out as potential miRNA target sites because the variants are in proximity to the open reading frame of the MECP2 gene. The third unclassified variant, *93GA, is predicted to result in mismatches in the sites matching the seed region of a miRNA by TargetScan. The fourth unclassified variant, *55CG, is predicted to be outside miRNA target site by TargetScan. However, another *in Silico* program (www.microrna.org) predicted that *55C is located in a miRNA target site and *55CG results in a mismatch between the miRNA and its putative binding site. The possibility that the 3 UTR variants may strengthen or create miRNA binding site will also be explored. It should be noted that *in vitro* studies such as luciferase reporter assay are necessary to determine if a putative miRNA target sites is indeed a functional miRNA binding site and if a sequence variant in the target site may affect miRNA function.

Prader-Willi like phenotype in a boy with homozygous 1p31.3 microdeletion. *S. Jaillard[1,2], H. Journel[3], S. Odent[1,4], V. David[1,5], L. Pasquier[1,4], C. Bendavid[1], J. Mosser[1], C. Henry[2], J. Lucas[2], C. Dubourg[1,5]* 1) Faculty Medicine, CS34317, UMR 6061, Rennes, France; 2) Histology Cytogenetics department, Pontchaillou University Hospital, Rennes, France; 3) Genetics department, CHBA Vannes, France; 4) Genetics department, Hopital Sud University Hospital, Rennes, France; 5) Molecular Genetics department, Pontchaillou University Hospital, Rennes, France.

We report a case of homozygous 1p31.3 microdeletion, identified by CGH-array (Agilent 4x44K), in a boy with early-onset severe hyperphagia, mental retardation and some dysmorphic features. The microdeletion has a minimal size of 80 kb and includes 3 genes *(DNAJC6, LEPROT, LEPR)*. The deletion homozygosity could be explained by uniparental disomy (isodisomy) or heterozygous deletion in both parents. The latter hypothesis was confirmed by multiplex PCR method although pedigree of the family apparently didnt show consanguinity. *LEPR* gene encodes the leptin receptor, which allows leptin to regulate adipose-tissue mass through hypothalamic effects on satiety and energy expenditure. Deleterious mutations of *LEPR* have been reported in subjects with early-onset severe obesity, hyperphagia, hypogonadotrophic hypogonadism, but without mental retardation and dysmorphic features. Heterozygous subjects do not present with obesity and have normal sexual development. *LEPROT* is linked to *LEPR* as it negatively controls *LEPR* function. Finally, *DNAJC6* could possibly explain mental retardation and dysmorphic features in our patient since this gene has a strong cerebral expression.

Combining information from candidate genes for autism in a risk score model improves predictive power: Statistical assessment of 15 genes and potential clinical applications. *J. Hager, M. Letexier, F. Rousseau, F. Tores*
Res & Development, IntegraGen SA, Evry, France.

Autism is a complex disorder with a strong genetic etiology. Although association with many genes has been reported, the modest increase in risk for each gene individually has hampered replication of association and renders the clinical use as a risk assessment tool ineffective. However, a multigene risk assessment model might provide more useful risk prediction information. We explored the impact on disease risk of 15 genes previously shown to be associated with autism: ASMT, ATP2B2, CNTNAP2, EN2, ITGB3, MARK1, MET, OXTR, PITX1, PRKCB1, SLC6A1, SLC6A11, SLC6A4, SLC6A7 and SLC25A12. To enhance chances for replication for single genes we genotyped tagSNPs at r2= 1.0 including SNPs reported associated previously. The 15 genes were genotyped in 493 families from AGRE (858 affecteds; male/female ratio 4:1). FBAT analysis was used to assess single gene associations for all families combined or for families with female affecteds and male affecteds only. After correction for multiple testing only ATP2B2, CNTNAP2, EN2, MARK1, PITX1, PRKCB1, SLC6A7 and SLC6A11 remained positive (p-value range p= 4.2x10-3 to p= 5.6x10-5). Sub-group analysis in families by sex of the affected children increased the evidence for association for some of these genes significantly. The average odds ratios for single genes were ~1.4. We then combined positive genes in a risk score to determine if this would increase predictive values for autism. Logistic regression adjusting for gender was used to determine odds ratios associated with increasing numbers of risk alleles. The risk for autism increased with the number of risk alleles in the combined genes (increase per risk allele app. 1.35). A ROC curve was constructed and the area under the curve (AUC) was tested for significance to describe the overall performance of the combined genes. The AUC of the corresponding ROC curve was approximately 0.7. Thus we demonstrate that combining information from several autism-related genes significantly increases the odds ratios and the power to assess the risk to develop autism in these families.

Development of psoriasiform skin lesions under anti-TNF therapy: a genetic link? *I. Cleynen[1], B. Claes[2], H. Nuytten[3], K. Van Steen[4], L. Henckaerts[1], M. Noman[1], H. Fidder[5], H. Cuppens[3], D. Lambrechts[2], P. Rutgeerts[1], S. Vermeire[1]* 1) Gastroenterology, KU & UZ Leuven, Belgium; 2) Transgenic Technology and Gene Therapy, KU Leuven, Belgium; 3) Center of Human Genetics, KU Leuven, Belgium; 4) Inst.Montefiore & Bioinformatique, ULG, Liège, Belgium; 5) Gastroenterology, Leiden University Medical Center, Netherlands.

Anti-TNF therapy is very efficacious for patients with inflammatory bowel disease (IBD). Despite an overall favorable safety profile, 21.7% of IBD patients treated with infliximab (IFX) in our hospital develop skin lesions, including psoriasiform dermatitis. Since there is no clear association between occurrence of skin lesions and cumulative dose of IFX, we hypothesized that certain genetic risk factors, described for both IBD and psoriasis (*IL23R* and *IL12B* variants, *Beta-defensin* (*DEFB4*) copy number (CN)) predispose to the occurrence of skin lesions in IFX-IBD patients. IFX-IBD patients (n=714) and healthy controls (n=342) were genotyped for 22 *IL23R* and 10 *IL12B* markers using the Sequenom platform. A subset of patients (n=258) was also tested for the number of *DEFB4* repeats, using qRT-PCR. All IFX-IBD patients were reviewed for occurrence of skin lesions. Analysis of genotype frequency differences between patients and controls could confirm *IL23R*, but not *IL12B* as a susceptibility gene for IBD. Multifactor Dimensionality Reduction, to test single and multilocus genetic combinations for the ability to predict the development of skin lesions, suggested that the best multilocus model consisted of 4 loci, with balanced accuracy of 63.1% and cross-validation consistency of 70%. Of the patients tested for the number of *DEFB4* copies, 27.5% developed skin lesions. The distribution of the CN was significantly different between patients who developed skin lesions compared to those who do not (Chi^2 p=0.03). Presence of 4 copies was associated with a significantly higher risk of developing skin lesions (Chi^2 p=0.01). The results of our study suggest that a common genetic predisposition may trigger the development of (psoriasiform) skin lesions in IBD patients treated with IFX.

Polymorphisms in the vesicle-associated membrane protein-associated protein A (VAPA) gene on chromosome 18p and bipolar disorder. *F. W. Lohoff, A. E. Weller, P. J. Bloch, A. H. Nall, T. N. Ferraro, W. H. Berrettini* Dept Psychiatry, Univ Pennsylvania, Philadelphia, PA.

Objectives: Linkage studies in bipolar disorder (BPD) suggest that a susceptibility locus exists on chromosome 18p11. The vesicle-associated membrane protein-associated protein A (VAPA) gene maps to this region. VAPA interacts with presynaptic proteins and is necessary for vesicular neurotransmission. Dysregulation of synaptic neurotransmission might contribute to the pathophysiology of BPD. In this study, we hypothesize that genetic variations in the VAPA gene contribute to BPD. Methods: Genotyping of 6 SNPs (rs494015; rs29193; rs29162; rs29145; rs29067; rs29066) was carried out in BPD patients (n=570) and healthy controls (n=730). Genotypes and allele frequencies were compared between groups using Chi square contingency analysis. Linkage disequilibrium (LD) between markers was calculated and estimated haplotype frequencies were compared between groups. Results: Single marker analysis revealed an association of rs29067 and rs29066 with BPD; however, after permutation correction, only rs29066 showed a trend towards an allelic association (p=0.066). Haplotype analysis did not show any significant association with disease after permutation correction. Conclusion: Our results provide suggestive evidence of an association between SNPs in the 3UTR of the VAPA gene and BPD. Interestingly, these SNPs are in close proximity to the microsatellite marker D18S464, which showed significant signals in previous linkage studies of BPD. Additional studies are necessary to confirm and elucidate the role of VAPA as a susceptibility gene for BPD on chromosome 18p.

Significant Results of CNV Analysis of Myopia in Schoolchildren. *A. Dellinger[1], T. L. Young[1,3], M. Seielstad[2], L. K. Goh[3], S. M. Saw[4,5], Y. J. Li[1]* 1) Ctr Human Genetics, Duke Univ Medical Ctr, Durham, NC; 2) Genome Inst. of Singapore; 3) Duke Singapore Graduate Med School; 4) Dept of Community, Occupational, and Family Med, Nat. Univ. of Singapore; 5) Singapore Eye Research Institute.

The Singapore Cohort study Of the Risk factors for Myopia (SCORM) followed the ocular development of over 1000 Singapore schoolchildren over several years. Illumina 550K SNP arrays were used to analyze the copy number variation (CNV) content of 1027 SCORM samples, of which 123 were normal, 109 were hyperopic, 730 were myopic, and 65 had unknown phenotype. We classified phenotype as: hyperopia (>0.50 diopters (D)), normal (-0.5 < D <= 0.5), low myopia (-3.00 < D <= -0.5), medium myopia (-6.0 < D <= -3.0), and high myopia (D <= -6.0) at the most recent visit. Nexus CGH was used to detect CNVs in the samples. Plink was used to perform tests of association. There is a trend of increasing numbers of CNVs per individual with increase in myopia severity- 33.9, 36.7, 37.8 for low, medium and high myopia cases, respectively, compared with 28.3 for controls (normal vision). Searching for CNVs of greater frequency in high myopia cases (n=93) than in controls (n=123) at uncorrected $p < 0.05$ showed: CNVs, including a gene, in MYP4 and MYP10 myopia loci; a CNV in a region implicated in glaucoma; and four genes involved in neurological diseases. Comparing all myopia cases to controls, CNVs were overrepresented ($p < 0.05$) in controls in myopia loci MYP3, MYP5, MYP6, MYP10, and MYP14. Genes in these CNVs primarily have neurological and developmental roles. Another gene involved in retinitis pigmentosa and retinal degeneration is significant in myopia when comparing high myopia vs. controls and medium+high myopia vs. controls. Deletion CNVs cover 156Kb of this gene. The region of the CNV with the most significant p-value ($p < 0.004$) is present in 7.5% of high myopia cases, 3% of both medium and low myopia cases, and 0% in controls. Use of additional CNV detection methods and qPCR to confirm these CNVs are warranted.

Combined malonic and methylmalonic aciduria (CMAMMA) with normal malonyl-CoA decarboxylase (MCD) activity detected by newborn screening (NBS). *R. Gavrilova[1], S. Tortorelli[2], K. Schoonderwoerd[3], D. Matern[2], P. Rinaldo[2], D. Gavrilov[2]* 1) Dept Medical Genetics, Mayo Clinic, Rochester, MN; 2) Biochemical Genetics Laboratory, Mayo Clinic, Rochester, MN; 3) Dept Clin Genet, Erasmus MC, Rotterdam, Netherlands.

Background: Malonic aciduria has been reported in <30 patients, most with deficiency of MCD. Few patients have been categorized as CMAMMA with normal MCD activity. Both conditions are associated with variable phenotypes ranging from severe neonatal crisis to developmental delays/neurologic abnormalities. No cases thus far are reported with persistent CMAMMA and no associated symptoms. Case report: We present a case of CMAMMA. Patient was detected by NBS showing elevated malonylcarnitine (0.97 mol/L; cutoff 0.20, 99%ile of normal population 0.09). Accumulation of 3-OH octanoylcarnitine was unlikely because follow up testing revealed malonic aciduria (>500 mmol/mol Cr; reference <5) combined with mild methylmalonic aciduria (21-197 mmol/mol Cr; reference <3.6). In vitro MCD activity was normal (13 nmol/hr/mg protein; reference >5.7). MLYCD gene sequencing encoding MCD revealed no mutations in patient or parents. Patient on treatment from birth with MCT oil, cholesterol,avoiding fasting, remains asymptomatic at age 12 months. Mother has elevated plasma malonyl carnitine (0.29 mmol/L, controls <0.13), but no elevated urine malonic/methylmalonic acid excretion. Conclusions: This is the first case of CMAMMA identified by NBS. Like most acylcarnitine species, malonylcarnitine is not specific marker for single condition, but requires at least differential diagnosis between two conditions. In contrast to published cases, our patient has no symptoms at 12 months. Patients mother is also asymptomatic with elevated malonyl carnitine in plasma. These findings suggest asymptomatic individuals with malonic aciduria caused by mutations in yet unidentified gene(s) can be detected by NBS. Clinical significance of CMAMMA and its underlying defect remain to be elucidated.

MicroRNAs affect mRNA expression in a range of cells during retinal development. *D. A. C. Simpson, A. Arora, J. Guduric-Fuchs* Centre for Vision Science, Queen's University Belfast, Belfast, Northern Ireland, United Kingdom.

MicroRNAs (miRNAs) are small RNA molecules (22 nucleotides) which have been shown to play an important role in both development and in maintenance of adult tissue. Conditional inactivation of miRNAs in the eye causes loss of visual function and progressive retinal degeneration. In addition to inhibiting translation, miRNAs can mediate degradation of targeted mRNAs. We have previously shown that the resulting signature of those miRNAs which are active in a tissue can be detected within mRNA expression profiles detected by microarray analysis. The purpose of this study was to predict which miRNAs are active during retinal development and in the adult retina and to confirm their expression.

Microarray expression data from the retinal stem cells (RSCs), developing and adult retina were generated or downloaded from public repositories. Analysis of gene expression profiles detected the effects of multiple miRNAs at each time point. The expression of all 20 selected miRNAs was confirmed by RT-PCR and the cellular distribution of representative candidates analyzed by in situ hybridization. Highly expressed miRNAs (eg. miR-124, miR-204, let-7, miR-9) showed strongest correlation with the predicted effects upon gene expression.

This study has detected the effect of miRNAs upon mRNA expression within the developing and adult retina. The validity of these observations is supported by the experimental confirmation of candidate miRNA expression. Identified miRNAs are likely to be important in retinal development and function. Sequence variants which caused misregulation of these miRNAs would be likely to contribute to retinal degeneration and disease. Conversely, manipulation of the expression of these miRNAs could potentially be used as a therapeutic tool in the future.

Duplication of Xq28 including the *MECP2* gene in a female with mental retardation and seizures. *D. N. Abuelo[1,4], N. Shur[1,4], S. R. Gunn[2], D. Mandelbaum[3,4]* 1) Dept Pediatrics, Rhode Island Hosp, Providence, RI; 2) Combimatrix Molecular Diagnostics, Irvine, CA; 3) Dept Neurology, Rhode Island Hosp, Providence, RI; 4) Warren Alpert School of Medicine at Brown University, Providence, RI.

MECP2 duplications have been associated with an X-linked syndrome characterized by severe mental retardation, progressive neurological symptoms and recurrent respiratory infections in males. Phenotypic features are nonspecific except for hypotonic, open mouth facies. Neurologic examination often shows axial hypotonia and spastic diplegia. Many of these patients die of pulmonary infections in infancy or early childhood. Our patient was born after a pregnancy complicated by a flu-like illness during the first trimester. Labor and delivery were unremarkable and she was thought to be a normal newborn. However psychomotor development was delayed from infancy. At age 1, she developed hemophagocytic lymphohistiocytosis and was treated with chemotherapy. Sequencing of the perforin gene did not reveal abnormalities. Head measurements stayed on the 10th percentile until 6 months of age, decreased to the 5th percentile at 12 months and subsequently have been in the microcephalic range. A Nissen fundoplication was done because of reflux and she received nutrition by gastrostomy tube from age 18 months to 3 years. Myoclonic seizures began at age 6 1/2. MRI showed white matter atrophy. Physical examination at age 7 showed severe microcephaly, no definite dysmorphism, axial hypotonia and lower extremity spasticity. Evidence of neurologic regression includes loss of a few words spoken at age 6 and loss of ability to walk with a walker that had been accomplished at age 3. Seizure frequency has been increasing and together with a higher dose of antiepileptic drugs, may contribute to the apparent regression. Array CGH showed a 4.0 Mb duplication of Xq28, including the *MECP2* gene. Previous reports of *MECP2* duplications have only involved male patients. Female carriers have been normal due to highly skewed X-inactivation. Our patient's X inactivation studies were normal, which correlates with her clinical findings. The reason for her random inactivation is not clear and is being investigated.

Partial rescue of the Kit-deficient testicular phenotype in *TSPY*-W^v/W^v mice. *S. Schubert[1], A. Schöner[1], I. Adham[2], W. Engel[2], J. Schmidtke[1]* 1) Institute of Human Genetics, Hannover Medical School, D-30625 Hannover, Germany; 2) Institute of Human Genetics, University of Göttingen, D-37073 Göttingen, Germany.

Human TSPY (**t**estis-**s**pecific **p**rotein, **Y**-encoded) is the product of a Y-chromosome-specific gene family located within the male specific region of the Y chromosome (MSY). *TSPY* expression is restricted to the testis where expression is limited to spermatogonia, spermatocytes and round spermatids. The *TSPY* expression pattern in germ cells and its homology to members of the TTSN-family indicate that TSPY functions as a proliferation factor of germ cells. In contrast to bovines and primates, where *TSPY* is organized as a repetitive gene family, the laboratory mouse harbours a single-copy pseudogene. We restored Tspy activity in a *TSPY* transgenic NMRI mouse line that carries a human *TSPY* transgene of approximately 50 copies on the mouse Y chromosome. In this study, we generated *TSPY* transgenic W^v/W^v mice and analyzed the histology of their testes and epididymides in order to contribute to understanding TSPY function in spermatogenesis. The Kit receptor, and its ligand stem cell factor (SCF) play a fundamental role in hematopoiesis, melanogenesis and gametogenesis. Homozygous W^v mutant male mice on a C57BL/6 background with a mutation on c-kit are infertile because of the almost total loss of germ cells in testes. We examined the testes of 58 adult *TSPY*-W^v/W^v males and 38 age-matched controls (NMRI-W^v/W^v) histologically. Round and/or elongated spermatids were observed in the testes of 35 out of 58 *TSPY*-W^v/W^v males and in 18 out of 38 NMRI-W^v/W^v mice. Sperms were identified in the epididymides of 23 out of 58 *TSPY*-W^v/W^v mice and in 3 out of 38 investigated NMRI-W^v/W^v males ($P<0.01$). We observed fertility in 4 out of 28 *TSPY*-W^v/W^v male mice mating with wild-type females, whereas none of the controls (18 NMRI-W^v/W^v males) produced offspring. Taken together our findings show that TSPY is able to partially rescue spermatogenesis in W^v/W^v mutants, an effect that is influenced to some extent by the NMRI genetic background.

Greater Burden of Rare Copy Number Variants in Schizophrenia. *J. Stone[1], Int. Schizophrenia Consortium[1,2,3,4,5,6,7,8,9]* 1) Psych & Neurodev Gen, Ctr Human Gen Res/MGH, Boston, MA.; The Stanley Center, The Broad Inst. of Harvard and MIT, Cambridge, MA, USA; 2) Inst. of Med. Sci., Univ. of Aberdeen, Aberdeen, UK; 3) Inst. of Molec. Med., TCD, Dublin, Ireland; 4) Dept. of Psychol. Med., Cardiff Univ., Cardiff, UK; 5) Windeyer Inst. of Med. Sci., UCL, London, UK; 6) Div. of Psych., Univ. of Edinburgh, Edinburgh, UK; 7) Dept. of Med. Epidemiology and Biostat., Karolinska Inst., Stockholm, Sweden; Dept. of Psych., UNC, Chapel Hill, NC; 8) Dep. of Psych. and Behavioral Sci., USC, Los Angeles, CA; 9) Queensland Inst. of Med. Res., Brisbane, Australia.

Schizophrenia (SCZ) is a severe mental disorder marked by hallucinations, delusions, cognitive deficits and apathy with heritability estimated at 73-90%. Inheritance patterns are complex and the number and type of genetic variants involved are not understood. Copy number variants (CNVs) have been identified in individual SCZ patients and in other neurodevelopmental disorders, but large-scale genome-wide surveys have not been performed. We report such a genome-wide survey of rare CNVs in 3,391 patients with SCZ and 3,181 ancestrally-matched controls using high-density microarrays. For CNVs that were observed in less than ~1% of the sample and greater than 100kb in length, the total burden is increased in SCZ patients compared to controls (P=3x10^{-5}; 1.151 fold increase). This effect was more pronounced for rarer, single-occurrence CNVs and for those that involved genes (P=5x10^{-6}) as opposed to those that did not (P=0.16). As expected, deletions were found within the region critical for velo-cardio-facial syndrome (P=0.0017, odds ratio (OR) =21.6), which includes psychotic symptoms in 30% of patients. Associations with SCZ were also found for large deletions on chromosome 15q13.2 (P = 0.0029, OR =17.9) and 1q21.1 (P = 0.0076, OR =6.6). These associations were not previously reported in the literature and remained significant after genome-wide correction. Overall, our results provide strong support for a model of SCZ pathogenesis that includes the effects of multiple rare structural variants, both genome-wide and at specific loci.

Symmetry of metabolic network. *Y. Xiao[1], H. Dong[2,3], W. Wang[1], L. Jin[2,4], M. Xiong[2,3]* 1) Department of Computing and Information Technology, Fudan University,ShangHai 200433, China; 2) School of Life Science, Fudan University, Shanghai 200433, China; 3) Human Genetics Center, University of Texas School of Public Health, Houston, TX 77225, USA; 4) Chinese Academy of Science-Max-Planck-Gesellschaft Partner Institute for Computational Biology, Shanghai Institutes for Biological Science, CAS, Shanghai, 200433, China;.

Previous studies on metabolic works mainly focus on the statistic properties of networks, including.the small world, power-law distribution of vertex degree, bow-tie model, building block of network motifs and hierarchical structure of the network topology. Symmetry has been shown to be universal across various empirical network systems, however rarely been studied so far. In this paper, we systematically investigate the symmetry in metabolic networks. Symmetry in directed graph is introduced and an algorithm to calculate symmetry in directed graphs is proposed. Several symmetry based indices are used to measure the degree of symmetry and heterogeneity of metabolic networks. We found that metabolic network is strong locally symmetric and weakly globally symmetric; and that the more advanced the species; the lower the degree of symmetry is and the larger the network heterogeneity is. We also calculate all the automorphism partitions and analyze the functional similarity among vertices in the same orbits. We found that compounds in the same orbit, i.e. vertices that are automorphic equivalent to each other, show much more similarity in function than compounds belongs to different orbits. Our study suggests that symmetry play an important role in the function and evolution of metabolic networks.

TMEM70 is a novel factor of mitochondrial ATPase biogenesis and its mutations cause isolated enzyme deficiency and neonatal encephalo-cardiomyopathy. *A. Čížková[1,2], V. Stránecký[1], J. A. Mayr[3], M. Tesařová[4], V. Havlíčková[2], J. Paul[2], R. Ivánek[1], AW. Kuss[5], H. Hansíková[4], V. kaplanová[2], M. Vrbacký[2], H. Hartmannová[1], L. Nosková[1], T. Honzík[4], Z. Drahota[2], M. Magner[4], W. Sperl[3], J. Zeman[4], J. Houštěk[2], S. Kmoch[1]* 1) Institute of Inherited Metabolic Disorders, Prague; 2) Institute of Physiology, ASCR, Prague; 3) Department of Pediatrics, Paracelsus Medical University, Salzburg; 4) Department of Pediatrics, 1st Faculty of Medicine, Prague; 5) Max Planck Institute for Molecular Genetics, Berlin.

ATP synthase defects of the nuclear genetic origin are characterized by selective decrease of the ATP synthase complex and a profound loss of both, synthetic and hydrolytic activities. With an exception of one case with ATP12 mutation no other affected nuclear genes have been identified yet. To identify the genetic defect in other patients we used Affymetrix 250K arrays, genotyped 8 index patients, their healthy siblings and parents from 6 families and intersected the mapping information with gene expression changes observed in patient fibroblasts (Agilent 44kArrays). The analysis illuminated a single gene, TMEM70, as it has been localized in a shared homozygosity region on chromosome 8, showed reduced transcript amount in patient fibroblasts and was characterized as a putative mitochondrial protein. Sequence analysis identified in patients a homozygous substitution c.317-2AG, located in the splice site of intron 2 of the TMEM70 which leads to aberrant splicing and loss of TMEM70 transcript. Screening of other suspected cases investigated in our institutions revealed 23 homozygous patients. In a single heterozygous patient a mutation c.118_119insGT encoding a truncated TMEM70 protein - p.Ser40CysfsX11 was identified on the second allele. Complementation of patient cell lines with TMEM70 restored biogenesis and metabolic function of the enzyme complex. Phylogenetic analysis revealed TMEM70 homologues in genomes of multicellular eukaryotes and plants, but not in yeast and fungi. We conclude, that TMEM70 is a novel factor of the ATPase biogenesis in higher eukaryotes and its defect is frequent among patients, particularly gypsies, with isolated ATPase deficiency.

Polymorphisms in Oxytocin Receptor and CD38 genes and Autism: Association and Functional Studies. *K. E. Tansey, M. J. Hill, R. J. L. Anney, M. Gill, L. Gallagher* Neuropsychiatric Genetics, Trinity College Dublin, Dublin, Ireland.

The neuropeptide oxytocin has recently been implicated in the aetiology of autism. It has effects on behaviour in relation to social cognition, compassing social memory formation, social recognition, and social motivation. Oxytocin is modulated by the oestrogen sex hormones, which heightens its candidacy for a role in autism where the ratio of affected males to affected females is highly skewed (4:1). CD38 plays a critical role in maternal nurturing behaviour and social recognition by regulating oxytocin secretion; implicating this gene in the aetiology of autism via the oxytocin system. We tested for association of oxytocin receptor (OXTR) using 20 tagging SNPs and 14 tagging SNPs for CD38 in 179 simplex families from the Irish Autism Study. We followed up genetic association studies with allelic expression imbalance (AEI) testing for alterations in expression of the OXTR and CD38 genes. Using lymphoblast cell lines from the CEU HapMap collection, we examined the influence of common variation and different levels of -Estradiol and Progesterone on AEI. Associations between OXTR and autism were found with 3 markers (rs11720238 p=0.031; rs7632287 p=0.0076; rs4564970 p=0.0091). Two SNPs showed association with a high functioning subset after multiple permutation testing (rs11720238 corrected p= 0.025; rs7632287 corrected p=0.0042). Associations between CD38 and autism were found with rs7655635 (corrected p=0.017). We observed AEI of OXTR and CD38 transcripts. The variation in OXTR was driven, in part, by a SNP in intron 3 (rs237897; p=0.0265). rs237897 was not associated with autism in our sample. The addition of hormones did not appear to alter AEI significantly from the baseline. These results confirm the importance of the oxytocin system in the aetiology of autism and identified SNPs involved in differential allelic expression.

Functional interactions of conserved non-coding (CNCs) sequences using circular chromosome conformation capture (4C). *D. Robyr, M. Friedli, G. Duriaux-Sail, S. E. Antonarakis* Genetic Medicine and Development, University of Geneva Medical School, Geneva, Switzerland.

The comparison of human chromosome 21 (Hsa21) sequences with the mouse syntenic regions led to the identification of roughly 3500 regions displaying an identity of >70% over a length of a least 100 nucleotides of ungaped alignment. About 65% (~ 2300) of these are conserved non-coding sequences (CNCs). Very little is known about the function of most CNCs. We speculated that a functional CNC might interact with its genomic target (i.e. an enhancer would bind to its cognate gene promoter). Thus, the identification of any part of the genome that interacts directly with a CNC could provide clues on the function of the latter. We have generated libraries of CNC-interacting DpnII fragments by chromosome conformation capture (4C) whose identity is determined by subsequent high-throughput sequencing. As proof of principle we have generated interacting maps between the human -globin locus control region (Dnase I hypersensitive sites 5, HS5) and the rest of the genome in the erithroïd K562 cell line. We have confirmed previous observations showing an interaction between HS5 and the promoter region of the gamma globin genes HBG1 as well as with the 3HS. Moreover, additional interactions were mapped to various Dnase I hypersensitive sites surrounding the -globin gene locus. We are currently screening for the interactions of 10 CNCs located in the two ENCODE regions of HSA21 in different cell lines. Preliminary results in K562 cells indicate that CNCs are capable of interactions with loci not only in cis and over several Mb, but also in trans with loci located on other chromosomes (between 1.2% and 82.2% of the sequenced tags are associated in trans depending of the CNC analysed). Interestingly, 4 CNCs located over the span of 250 kb show interactions with the CNS specific genes Olig1 and Olig2. Injection in fertilized mouse oocytes of two of these CNCs using a lentiviral vector drives the expression of a lacZ reporter gene in the somites and brain at E11 in embryos, reminiscent of olig expression.

Establishment of the Undiagnosed Diseases Program at the National Institutes of Health. *C. E. Wahl, M. E. Nehrebecky, W. Gahl* Office of Rare Diseases, NHGRI/NIH, Bethesda, MD.

The National Institutes of Health, through the National Human Genome Research Institute and the NIH Office of Rare Diseases announces the establishment of the Undiagnosed Diseases Program (UDP). Referrals are accepted from medical providers with patients deemed undiagnosed after an adequate workup. The NIH UDP screens arriving referrals to generate a subset of patients to be invited to the NIH for further workup and evaluation. The stated goals of the UDP include providing answers to patients with mysterious conditions that have long eluded diagnosis and advancing medical knowledge about rare and common diseases. Each patient visiting the NIH is evaluated by a custom-designed multidisciplinary team based on their presenting illness and the results of prior medical evaluation. Participating specialists span the expertise of the NIH community including rheumatology,oncology, mental health, nephrology, hematology, ophthalmology, neurology, laboratory medicine, pain and palliative care, bone disorders, endocrinology, oncology, immunology, dermatology, primary immunodeficiency, dentistry, genetics, pathology, pulmonology, cardiology, primary immunodeficiency, internal medicine, pediatrics and hepatology. Medical data collected during the evaluation is returned to the referring provider regardless of whether a definitive diagnosis was achieved during the visit. In addition to the potential for diagnosis, participating patients may benefit from additional ideas for treatment of ongoing medical problems. Data from the patient evaluations is be used to generate ideas and hypothesis for continuing medical research. Since the announcement of the program on May 19, 2008, approximately 400 inquires have been received regarding potential participation. Interested medical providers and their patients may obtain more information from the program by visiting http://rarediseases.info.nih.gov/UndiagnosedDiseases/FAQ.aspx.

Massive Parallel DNA (re)Sequencing in Hypertrophic Cardiomyopathy: a powerful approach to detect substitutions and indels. *J.-L. Blouin[1], C. Iseli[2,3], D. Robyr[4], A. Munoz[4], S. E. Antonarakis[1,4], S. Fokstuen[1]* 1) Dept Genetic Medicine, Univ Hosps Geneva, Geneva, Switzerland; 2) Ludwig Institute for Cancer Research, Lausanne, Switzerland; 3) Swiss Institute of Bioinformatics, Lausanne, Switzerland; 4) Genetic Medicine and Development, University of Geneva School of Medicine, Switzerland.

With the prevalence of 1/500, Hypertrophic Cardiomyopathy (HCM) represents the most common inherited cardiovascular disorder, also considered as the primary cause of sudden cardiac death in young adults. More than 450 different pathogenic mutations in at least 16 genes have been identified. In order to overcome this extensive genetic heterogeneity we had developed a 30 Kbp HCM-custom-DNA-resequencing-array (HCM-RA) comprising all exons (n=160), splice-sites and 5-UTR of 12 HCM genes (Fokstuen et al, 2008). Although very efficient, this approach did not detect small indels, accounting for 14% of known HCM mutations. Moreover, the HCM-RA lacks flexibility since gene additions require a new design. In order to solve these shortcomings we assessed the performance of the developing Massive Parallel DNA Sequencing (MPS) technology. We reanalysed these regions (PCR amplified) of 12 genes in a total of 19 patients, previously hybridized on the HCM-RA (11 without known mutations, 8 positive-controls as a composite-pool), in a single channel of a Illumina-Solexa sequencer. Every single base (27070 bases, 570 fold coverage) was analyzed using a newly developed data analysis pipeline on the Vital-IT HPC platform. All the 8 known pathogenic mutations and the 18 SNPs previously identified by HCM-RA were also observed in MPS. Furthermore we found new indels (c.1028delC/p.Thr343fsX349, c.506-12delC in MYBPC3; c.53-15_-11delTTCTC in TNNT2) which were confirmed by classical sequencing. Interestingly we observed a complex nucleotide change altering the acceptor splice site of intron 20 of MYBPC3 [c.2146-9C>A+c.2146-2delA]. Although there is still a need for improvement in target enrichment, and data analysis, MPS hold considerable promises in DNA mutation/variant analysis underlying highly heterogeneous or multigenic genetic disorders in clinical practice.

Ephrin B4 Receptor (*EPHB4*) gene polymorphisms and risk of intracranial hemorrhage in patients with brain arteriovenous malformations (BAVM). *S. Weinsheimer[1], H. Kim[1,2,3], L. Pawlikowska[1,2], P.-Y. Kwok[2], C. E. McCulloch[3], W. L. Young[1,4]* 1) Center for Cerebrovascular Research, Dept Anesthesia; 2) Institute for Human Genetics; 3) Dept Epidemiology and Biostatistics; 4) Dept Neurology and Neurological Surgery; Univ. California, San Francisco, CA.

Background: BAVM are a tangle of abnormal vessels directly shunting blood from the arterial to venous circulation and an important cause of intracranial hemorrhage (ICH). EPHB4 is involved in arterial-venous determination during embryogenesis; altered signaling could lead to vascular instability resulting in ICH. We investigated the association of single-nucleotide polymorphisms (SNPs) and haplotypes in *EPHB4* with risk of ICH at clinical presentation in BAVM patients. **Methods**: Eight haplotype-tagging SNPs (2 exonic, 4 intronic, and 2 intergenic) spanning 28kb were tested in 146 Caucasian BAVM patients (56 ICH and 90 non-ICH) using allelic, haplotypic, and principal components analysis (PCA). Associated SNPs were then genotyped in a second set of 102 cases (37 ICH and 65 non-ICH) and data combined for multivariable logistic regression. **Results**: The minor alleles of 2 intronic SNPs were associated with reduced risk of ICH presentation (rs314313 C, P=0.005; rs314308 T, P=0.0004). Haplotype analysis revealed a significant overall association (^{2}=17.24, 6 df, P=0.008), and 2 haplotypes containing the minor allele of rs314308 (GCCTGGGT, P=0.003; and GTCTGGGC, P=0.036) were also associated with reduced risk. Sliding windows of 3-SNP haplotypes excluded 2 SNPs at the 3 end. PCA results retaining 2 components explained 91% of the total variance (P=0.003), and complemented haplotype results by implicating the 4 SNPs at the 5 end, including rs314308 and rs314313. These 2 SNPs were validated in a second set, and the combined data resulted in greater significance (rs314313, P=0.0007; rs314308, P=0.00008); the SNP association persisted after adjusting for age, gender, BAVM size, deep venous drainage and eloquent location. **Conclusions**: *EPHB4* polymorphisms at the 5 end of the gene are associated with risk of ICH presentation in Caucasian BAVM patients warranting further study.

Genetic Characterization of Zimmerman Laband syndrome. *T. S. Han[1], P. S. Hart[2], P. Sulima[1], C. Turner[2], S. I. Jang[1], T. C. Hart[1]* 1) NIDCR, NIH, Bethesda, MD; 2) NHGRI, NIH, Bethesda, MD.

A 7 y.o. African American male with extensive gingival overgrowth and minor dysmorphic features, including shortened nasal columella, posteriorly rotated ears, and large patulous lips, was ascertained. Family history was negative for gingival overgrowth. Because of the dysmorphisms and maternal history of pregnancy loss, karyotype analysis was conducted, revealing 46, XY, t(3;17) (p21.1;q23.3. 3). Haplotype analysis demonstrated the translocation arose on the paternal homologues. The father does not have gingival overgrowth, indicating a de novo event. The clinical features in this patient are most consistent with Zimmermann-Laband syndrome (ZLS), a rare autosomal dominant disorder of unknown etiology. The most penetrant finding in ZLS is gingival overgrowth. Breakpoint mapping demonstrated the break on chromosome 3 occurred in intron 3 of the CACNA2D3 gene. The chromosome 17 break did not occur within a gene or highly conserved region. The der 17 revealed 21 nucleotides that could not be assigned to either breakpoint region and did not map with 100% homology to any chromosomal segment. Within this 21 bp is a 9 bp sequence that is present on chromosome 17 at the location of the breakpoint and may represent a duplication. Expression of genes flanking translocation breakpoints were evaluated in gingiva from the patient and controls. HESRG, MAP2K6 and ACTR8 expression were not found to be different by quantitative PCR. LRTM1 and KCNJ16 were not expressed in gingiva. RT- PCR using a Taqman probe spanning the exon 3-4 junction of CACNA2D3 demonstrated significantly decreased expression in patient gingiva. Decreased expression of CACNA2D3 appears to result in gingival overgrowth. This conclusion is supported by the observation of another patient with gingival overgrowth with a break in this same gene, (3 to the break in our patient) and by a mother/daughter with a break approximately 0.1 Mb 3 of CACNA2D3. These results implicate CACNA2D3 in gingival overgrowth and may provide clues into the drug-induced forms which also center around calcium metabolism.

Identification of the gene responsible for a congenital muscular dystrophy with hyperlaxity (CMDH) in a French-Canadian cohort. *M. Tetreault[1], I. Thiffault[1], L. Loisel[1], J. Mathieu[2], Y. Robitaille[3], M. Vanasse[4], B. Brais[1]* 1) Neuromics Center for Excellence of Université de Montréal, Université de Montréal, CR-CHUM Hôpital Notre-Dame, Montreal, QC, Canada;; 2) Carrefour de la Santé de Jonquière, Saguenay, QC, Canada; 3) Département de pathologie, Hôpital Sainte-Justine, Montreal, QC, Canada; 4) Clinique des maladies neuromusculaires, Centre de réadaptation Marie-Enfant, Hôpital Sainte-Justine Hospital, Montréal, QC, Canada.

Congenital muscular dystrophies (CMD) are a heterogeneous group of disorders. A growing number of CMD have been found to be associated with joint hyperlaxicity. We recruited 16 French-Canadian cases belonging to 13 families from Southwestern Quebec affected by a novel autosomal recessive congenital muscular dystrophy with hyperlaxicity (CMDH). All patients present muscle weakness, proximal contractures coexisting with distal joint hyperlaxicity. They have a more benign course than most described CMD, with preservation of walking into adult age in most cases. A genome wide scan of 500 microsatellites markers, allowed us to identify the chromosomal locus responsible for CMDH on chromosome 3 at position 3p23-21.3. Fine mapping to a 0.11Kb interval was performed by SNPs genotyping using the Sequenom iPlex platform. We uncovered complex sequence insertion mutations, including an Alu insertion, in the CMDH causative gene. The mutations lead to abnormal molecular weight CMDH mRNA and protein. The identification of mutations in a gene coding for a transmembrane protein suggest that as in other muscular dystrophies the integrity of the interactions between the extracellular matrix and the membrane are likely disturbed in this CMD with hyperlaxicity.

The influence of metabolic polymorphisms and smoking during pregnancy on fetal growth and preterm delivery.

C. L. Relton[1], R. M. Freathy[2], S. M. Ring[3], A. Groom[1], R. Gray[4], A. T. Hattersley[2], T. M. Frayling[2], G. Davey Smith[3] 1) Institute of Human Genetics, Newcastle University, Newcastle upon Tyne, United Kingdom; 2) Institute of Biomedical and Clinical Science, Peninsula Medical School, Exeter, United Kingdom; 3) Department of Social Medicine, Bristol University, United Kingdom; 4) National Perinatal Epidemiology Unit, Oxford University, Oxford, United Kingdom.

Exposure of the developing fetus to cigarette smoke is believed to compromise fetal growth and may trigger premature delivery. Evidence suggests that fetal growth and preterm delivery are influenced by the ability to detoxify the products of cigarette smoke and this in turn is influenced by genetic variation in the detoxification pathway. We performed a large association study using the Avon Longitudinal Study of Parents and Children (ALSPAC), genotyping 8,499 mothers and 8,617 children. The cohort included 1,660 children born small for gestational age (SGA;<10th centile birth weight) and 928 children born preterm (<37 weeks gestation). 30.7% of mothers smoked during pregnancy and 69.3% were non-smokers. We analysed common variants in 4 genes selected to tag known common functional and copy number variation; GSTM1 deletion, CYP1a1_2a (rs4646903), CYP1a1_2c (rs1048943) and UGT2b28 (rs11249532). A maternal copy of the UGT2b28 variant modestly increases risk of delivering a SGA infant in non-smokers (OR 1.26 [95%CI 1.04, 1.53] p=0.021) and this effect is exacerbated in smokers (OR 2.05 [95% CI 1.59, 2.64] p<0.001). A maternal copy of the UGT2b28 variant was associated with an increased risk of preterm delivery in non-smokers (OR 1.47 [95%CI 1.15, 1.87] p=0.002) but not in smokers. Smoking during pregnancy was associated with a small increase in risk of preterm delivery (OR 1.21 [95%CI 1.05, 1.39] p=0.01). None of the other variants studied influenced the risk of delivering a SGA or preterm infant in excess of that expected by smoking alone. Fetal development and gestation length are influenced by the presence of a common polymorphism in UGT2b28. This may be due to its function in detoxification or its role in androgen and estrogen metabolism.

Several microRNAs are involved in the susceptibility to eating disorders as detected by association studies using a comprehensive miRNA-targeted genotyping platform. *Y. Espinosa-Parrilla[1,2], M. Muiños-Gimeno[1,2], J. M. Mercader[1,3], M. Montfort[1,3], M. Bayés[1,3], M. Gratacòs[1,2], F. Fernández-Aranda[4], X. Estivill[1,2,3,5]* 1) Genes & Disease Program, Centre de Regulació Genòmica, Barcelona; 2) CIBERESP, CRG, Barcelona; 3) CeGen, CRG, Barcelona; 4) CIBEROBN, Psychiatric Service, Ciutat Sanitaria Bellvitge, LHospitalet; 5) Pompeu Fabra University, Barcelona, Spain.

Involvement of microRNAs (miRNAs) in the control of neuronal differentiation and synaptic plasticity suggests a role for these molecules in the aetiology of psychiatric disorders. In order to evaluate this hypothesis we performed association studies in eating disorders (ED) using SNPs in miRNA regions. We analysed the organization of 326 human miRNAs (MiRBase 7.1) and defined 164 regions (2Mb of genomic DNA) including miRNA sequences as well as their 5kb flanking regions. Exploration of the SNP coverage (dbSNP128) revealed a statistically significant lower SNP density in miRNAs than in their flanking regions (1.8 versus 3.4SNPs/kb, p0.005, F test). Further, we constructed a panel of 768 SNPs covering miRNA regions and performed association analysis after genotyping 340 Spanish controls and 294 patients with different types of ED (custom Golden Gate assay, Illumina). We found strong associations ($p8x10^{-4}$) for one miRNA with EDNOS (ED non Otherwise Specified), two other miRNAs with Bulimia Nervosa and a miRNA cluster with Anorexia Nervosa. To identify common genetic factors for ED we studied the intersections of nominal associations among the three sub-phenotypes and found 10 shared associations. Seven of these miRNAs were also associated with Minimum Body Mass Index and one brain expressed miRNA with age at onset ($p=8.0x10^{-5}$, significant after correction for multiple testing). TargetScan predictions indicated 101 genes to be potentially regulated by this miRNA. Interestingly, Ingenuity Pathways Analysis revealed an enrichment of genes involved in development and function of the endocrine as well as nervous system ($p=1.09E^{-5}$-$p=6.49E^{-3}$). All together indicates that miRNA mediated regulation could underlie the genetic susceptibility to these complex disorders.

A new locus for recessive autosomal arthrogryposis multiplex congenita. *R. Attali[1], N. Warwar[1], A. Israel[1], A. Laquerriere[2], H. Topaloglu[3], Y. Nevo[4], Z. Ben Neriah[1], J. Melki[1]* 1) Dept. of Human Genetics, Hadassah University Hospital, Jerusalem, Israel; 2) Dept. of Pathology, CHU de Rouen, France; 3) Dept. of Child Neurology, Hacettepe Children's Hospital, Ankara, Turkey; 4) NeuroPediatric Unit, Dept of Pediatry, Hadassah University Hospital, Jerusalem, Israel.

Arthrogryposis multiplex congenita (AMC) is clinically and genetically heterogeneous. AMC with hypokinesia or akinesia may be isolated or associated with additional features. AMC can be secondary to a myopathic or a neurogenic process, a connective tissue disorder, a mechanical cause or a maternal illness. The neurogenic forms characterized by the paucity of motor neurons associated with muscular atrophy are considered to be the most common form. In order to elucidate the genetic basis of recessive autosomal AMC (RA-AMC) of neurogenic origin, patients were recruited through the geneticists, neuropediatricans or fetopathologists based on strict clinical inclusion criteria. A total of 7 families were selected, 4 of them were consanguineous and/or with at least two affected children. No homozygous or heterozygous deletion of SMN1 exon 7 was found in the index cases excluding this locus. Whole genome analysis was performed using 250K snp microarray (Affymetrix). In a large consanguineous pedigree, linkage analysis revealed a highly candidate region of 6 Mb with a positive lod score of 2.85, the other regions of the genome giving a lod score below -2. In the linked region, the affected patients and obligate carriers were homozygous and heterozygous, respectively. Several genes have been recently identified as responsible for RA-AMC of neurogenic origin including SMN1, GLE1, ERBB3, PIP5K1C and UBE1. This region of Chromosome 6 linked to the disease locus in our family does not contain a gene known to be involved in arthrogryposis or in motor neuron diseases indicating that we found a new locus. Other families from the same geographic origin or with similar phenotype will be tested for this new locus. Sequence analysis of the most candidate genes found in this region should allow the identification of the gene responsible for this very severe disease.

Fine mapping an Autism Susceptibility locus on chromosome 1q23-24. *P. Garavito[1], N. Gharani[1], M. Azaro[1], CW. Bartlett[2], O. Stein[2], R. Goedken[2], J. Millonig[1,3,4], E. DiCicco-Bloom[4], VJ. Vieland[2], LM. Brzustowicz[1]* 1) Dept Genetics, Rutgers University, Piscataway, NJ; 2) Battelle Center for Mathematical Medicine, The Research Institute at Nationwide Children's Hospital & The Ohio State University, Columbus, OH; 3) Center for Advanced Biotechnology and Medicine; 4) Dept Neuroscience & Cell Biology, UMDNJ-RWJMS, Piscataway, NJ.

Multiple linkage and association studies in autism have suggested several loci that may harbor autism susceptibility genes but no universally accepted gene has emerged. One possible explanation for the lack of consistency of these studies is genetic heterogeneity. To address this issue, phenotypic subsetting of family data and the Posterior Probability of Linkage (PPL) statistic have been used by different studies. Previously, PPL analysis of the Autism Genome Resource Exchange (AGRE) genome scan data for 303 families identified a 30cM linkage region on chromosome 1q23-24, in a subset of 46 families with broad diagnosis and negative for Phrase Speech Delay (PSD-). Here we have fine mapped the 30cM linkage region through high density microsatellite genotyping. Multipoint PPL analysis of the genotype data narrowed the 30cM linkage interval to 5cM. Currently we are conducting a high density SNP genotyping and Linkage Disequilibrium (LD) mapping of this interval. We initially focused on a central region of 335kb that encompassed two evolutionarily ultra conserved regions. Data from the International HapMap Project was used to select 76 tagSNPs for this region. The posterior probability of linkage disequilibrium (PPLD) which directly measures the probability of association was used to evaluate LD between the SNPs and the disease locus. PPLD scores > 0.02 indicates evidence in favor of LD. So far PPLD data for 44 tagSNPs have not provided evidence for association. In addition, we analyzed 254 SNPs from the recently released AGRE Affymetrix 500K SNP array data from the chromosome 1 interval. 13 SNPs show PPLD values greater than 0.02 indicating possible association of these markers with the trait locus. rs2800785 provided the highest PPLD score of 0.13 and is in intron 2 of PBX1 suggesting PBX1 as a potential candidate gene for autism.

Diagnosis of neuronal ceroid lipofuscinoses. *N. Abdelmoula[1], S. Zekri[2], H. Jaafoura[2], A. Amouri[3], R. Louati[1], T. Rebai[1]* 1) Lab Histology, Univ Medicine, Sfax, Tunisia; 2) Lab electronic microscopy, Univ Medecine, Tunis, Tunisia; 3) Lab Histomogy and cytogenetics, Univ Medecine, Tunis, Tunisia.

The NCLs are a family of genetically inherited metabolic storage diseases that exhibit a common pathology, lysosomal accumulation of autofluorescent lipopigment, neurodegeneration and premature death. NCL is inherited in an autosomal recessive manner and mutations in at least seven genes, CLN1, CLN2, CLN3, CLN5, CLN6, CLN8 and CTSD (cathepsin D) are known to result in human disease. Infantile neuronal ceroid lipofuscinosis is caused by mutations in CLN1 at 1p32 chromosome, the gene encoding the enzyme palmitoyl protein thioesterase 1 (PPT1). The clinical course and age of onset of the disease vary much due to the different mutations in CLN1 ranging from infantile-onset devastating disease to an adult-onset form with symptoms presenting in the fourth decade. For the majority of families affected by one of the neuronal ceroid lipofuscinoses (NCLs), a biochemical and/or genetic diagnosis can be achieved. We report the observation of a child in whom diagnosis of CLN1 was difficult to confirm at the clinical level: age at onset of symptoms at 12 months, developmental delay, visual impairment, attention deficit, central hypotonia, seizures, jerks, motor dysfunction with spasticity of the limbs and progressive diffuse brain atrophy on MRI. Ultrastructural analysis performed in skin punch biopsy permit to show granular osmiophilic deposits (GROD) in the secretory eccrine sweat gland epithelial cells. Molecular screening of CLN1 and CLN2 is conducted but biochemical testing to study PPT1 activity was difficult to consider. We show through this observation and review of literature current methods available to achieve clinical, pathological, biochemical and genetic diagnosis in children presenting with symptoms suggestive of one of the NCLs and interest of such diagnosis for therapeutic options and genetic counselling with prenatal diagnosis.

Identification of the LGMD2M gene by SNP homozygosity mapping in a French-Canadian cohort. *V. Bolduc[1], J. Jarry[1], MF. Rioux[2], Y. Robitaille[3], I. Thiffault[1], M. Tetreault[1], MJ. Dicaire[1], L. Loisel[1], JP. Bouchard[4], B. Brais[1]* 1) CENUM, CRCHUM Notre-Dame, Universite de Montreal, Montreal, Quebec, Canada; 2) CHUS, Universite de Sherbrooke, Sherbrooke, Quebec, Canada; 3) Hopital Ste-Justine, Montreal, Quebec, Canada; 4) CHA-Hopital Enfant-Jesus, Universite Laval, Quebec, Canada.

Limb-girdle muscular dystrophies (LGMD) represent a heterogeneous group of disorders characterized by progressive proximal weakness and atrophy. We have identified French-Canadian patients displaying a new form of LGMD associated with prominent quadriceps atrophy and myalgia (LGMD2M). To identify the mutated gene, we took advantage of high-density single nucleotide polymorphism (SNP) microarrays and genotyped three affected sibs of a first-degree consanguineous family, using the Illumina HumanHap300 (9 kb resolution). Homozygosity analysis revealed a 4.5 Mb region on chromosome 11, located next to the chromosome 11p13-p12 interval previously identified by microsatellite linkage analysis. The lower resolution obtained with the microsatellite genotyping (8 cM) precluded the identification of this homozygous region. Genomic and cDNA sequencing of the genes lying in the 4.5 Mb interval uncovered an illegitimate exonic splice site mutation within the LGMD2M gene, producing a 38pb-deletion in the cDNA sequence predicted to lead to a premature stop codon. The function of the mutated protein is unknown but it is highly expressed in muscle. This study identified a new LGMD gene belonging to a protein family not previously identified to be mutated in a neuromuscular disorder.

Extended Fisher-Yates Haplotype Exact Tests to Detect Genetic Disequilibrium for Genome-wide Scans of Human Genome. *R. Fan[1], K. Lange[2], M. Zhong[1]* 1) Dept Statistics, Texas A&M Univ, College Station, TX; 2) Department of Human Genetics, David Geffen School of Medicine,University of California, Los Angeles, CA.

The concepts of Hardy-Weinberg equilibrium and linkage equilibrium are central in population genetics theory. Departure from the Hardy-Weinberg equilibrium and linkage equilibrium leads to genetic disequilibrium, which can be very useful. For instance, it can point to the association of particular alleles with an increased risk of disease, can reveal population evolution and population substructure, and can indicate positive selection. Thus, tests of genetic equilibrium are of fundamental importance in population genetics and in mapping genes of complex diseases that continues to plague us. With the advent of dense maps of human genetic variations, it is possible to test genetic equilibrium across the human genome. In this research, we develop a novel statistical method to perform genome-wide test of genetic equilibrium and local fine mapping of candidate gene regions. Extended haplotype exact tests based on multivariate Fisher-Yates distribution are developed to provide insights about structure of human genome. Robustness of the proposed methods is evaluated via type I error calculation and comparison. The methods are applied to the HAPMAP phase II haplotype data for both genome-wide scans and fine mapping of local candidate gene regions.

The contribution of common and rare Copy Number Variants to disease risk in seven common diseases. *M. Hurles on behalf of the Wellcome Trust Case Control Consortium* Wellcome Trust Sanger Inst, Cambridge, United Kingdom.

Recent SNP-based genome-wide association studies have confirmed that the majority of the heritability of most common diseases does not reside in common SNPs with large effect sizes. One source of the remaining genetic risk could be common CNVs whose effects are poorly captured by genotyped SNPs. Another potential source could be rare alleles of relative large effect sizes. Such rare alleles are likely to be drawn from all forms of genetic variation in the human genome, including SNPs, indels and structural variants. Efficiently identifying such rare alleles requires large sample sizes, and ascertainment of variation unbiased to allele frequency. The identification of CNV from the Affymetrix 500k SNP genotyping data in the ~17,000 individuals studied by the Wellcome Trust Case Control consortium provides an opportunity to assess the impact of both common and rare CNVs on disease risk. We identified over 200,000 CNVs in the seven disease collections and two case collections, which reside in ~9,000 non-redundant locations in the human genome. We will report the results from our association testing of 100 common CNVs against these seven diseases. We also sought genes, pathways and genomic locations enriched for rare CNVs in each disease, and identified many disease-specific CNVs with 0.25-0.5% frequency There are compelling biological interpretations for many of the genes enriched for CNVs in these diseases. We will raise issues about the prospects for validation and the level of evidence required for inference with rare variants.

CYP7B1 in non-consanguineous hereditary spastic paraplegia. *C. Beetz[1], R. Schule[2], E. Brandt[1], M. Khundadse[1], K. Karle[2], M. Synofzik[2], M. Auer-Grumbach[3], A. Crosby[4], C. Hubner[1], L. Schols[2], T. Deufel[1]* 1) Institute of Clinical Chemistry, University Hospital Jena, Germany; 2) Herthie Institute for Clinical Brain Research Tübingen, Germany; 3) Centre for Medical Research, Medical University Graz, Austria; 4) Department of Medical Genetics, St. Georgess University London, UK.

Hereditary spastic paraplegia (HSP) is a neurodegenerative condition affecting lower limb movement. Homozygous mutations in CYP7B1 have been identified in several consanguineous families that represented HSP type 5 (SPG5), i.e. one of the many genetic forms of the disease. We used direct sequencing and multiplex ligation-dependent probe amplification (MLPA) to screen apparently sporadic HSP patients (n=12) as well as index patients from non-consanguineous recessive (n=8) and from dominant families (n=8) for mutations in CYP7B1. MLPA indicated absence of copy number aberrations in all samples. One sporadic patient showing HSP as well as optic atrophy carried a homozygous nonsense mutation, whereas compound heterozygosity (frameshift + missense) was observed in a recessive family with a clinically pure phenotype. We also found a heterozygous missense alteration to segregate in a small dominant family and a novel coding SNP to be significantly associated with the presence of cerebellar signs. The unexpectedly high frequency of 10% (2 of 20) SPG5 in our cohort of recessive and apparently sporadic HSP patients as well as a potentially modifying role for CYP7B1 variants need to be confirmed by larger studies.

Dinucleotide repeat tracts in human gene promoters. *A. Jasinska[1], M. Mason[1], C. Sabatti[2], N. Freimer[1]* 1) Dept Psychiatry; 2) Dept Statistics and Dept Human Genetics, Univ California, Los Angeles, CA.

The widespread occurrence of polymorphic short tandem repeats (STRs) in the human genome make them an important source of inter-individual variation. STR polymorphism is involved in abnormal gene regulation in several human disorders. As intergenic regions are enriched in dinucleotide (DINT) repeats - known to be a highly polymorphic class of STRs and shown to have gene regulation potential - we investigated their divergence between human and chimpanzee as well as their co-localization with genomic features, to identify STRs potentially involved in human-specific traits. We searched human and chimpanzee genomic sequences for DINT tracts 12 repeats. We focused on tracts located in a core promoter region of human genes up to 1500 bp upstream and 500 bp downstream of genes. We analyzed altogether 37.2 Mb of human promoter sequence, identifying 649 distinct DINTs distributed at 4 times lower density compared to total genome sequence. Almost all tract types are significantly longer in human compared to their chimpanzee orthologs. Some repeat tracts with increased length might exceed a length threshold triggering higher mutability, length polymorphism, and possible gain of a regulatory role. The length of numerous DINT tracts oscillates close to the length predicting no or low length polymorphism in one species while in the other species the tract expanded and most likely exists as multiple alleles. Excessive tract length divergence (>15 units) was found in 9 human and 1 chimpanzee expanded tracts, with extreme examples being the FLJ10374 and C8orf31 human tracts which are more than 25 units longer than their chimpanzee orthologs. The most interesting tracts show putative polymorphism which could influence gene activity e.g. by affecting CpG islands (CpGIs), such as the long FCHSD2 and HMGA2 tracts completely contained within CpGIs. More than 40 DINTs considerably overlap CpGIs and at least two of them (SLC6A9 and HHIP) are highly polymorphic STRs. The promoter DINT tracts showing greatest divergence, polymorphism potential and localization in gene regulatory regions could be interesting candidate loci for individual variation in transcript levels.

Joint hypermobility, skin hyperelasticity and microcephaly: Ehlers-Danlos syndrome type VI, VII C or a new syndrome? *A. Perez, T. ZANOLLA, N. SOBREIRA, M. CERNACH* Centro de Genetica Medica, Unifesp - EPM, Sao Paulo, Brazil.

The Ehlers-Danlos syndromes (EDS) are a group of heritable connective tissue disorders characterized by joint hypermobility, skin extensibility, and tissue fragility. Genetic heterogeneity is a characteristic of EDS type VI and type VII C. EDS type VI (OMIM 225400) is an autosomal recessive disease caused by a deficiency of lysil hydroxylase (PLOD), characterized for generalized joint laxity, progressive scoliosis, scleral fragility and rupture of the ocular globe. EDS type VII (OMIM 225410), is also an autosomal recessive disease but caused by deficiency of procollagen I N-terminal peptidase, characterized for severe skin fragility. Both types may courses with microcephaly (Beighton et al., 1998). We describe a 4 years-old male born from a non consanguineous young couple. Delivery was pos-term by cesarean section. His examination showed microcephaly, triangular face, epicanthal folds, telecantus, large, cup shaped and hyperextensible ears, flat nasal bridge, thin superior lip, short neck, pterigium colli, pectus escavatum, generalized joint hypermobility, syndactyly of fingers, medial deviation of both halux, hyperlordosis, hip luxation, smooth, velvety and redundant skin and left criptorquia. He presents hypotonia and mild developmental delay. G-banding karyotype from lymphocytes cells was 46, XY, echocardiography and audiometry are normal, and microcitic hypochromic anemia. Skeletal survey documented bilateral hip luxation. Skin biopsy will be done. The most significant clinical characteristics of this patient are psychomotor retardation, microcephaly, joint hyperlaxity and hip luxation suggesting a connective tissue disorder like EDS type VI and type VII C but the presence of mental retardation, syndactyly of fingers, facial signs and speech abnormalities may rule it out. Nevertheless this patient resembles two brothers described by Mégarbané et al. at 2001 whose phenotype is characterized by microcephaly, rudimentary language, and syndactyly of fingers, hip dislocation and similar facial appearance and brain MRI abnormalities. We believe that this case is the third of the literature and can help on delineation of a new syndrome.

Genome-wide association study (GWAS) identifies novel SNPs associated with autism. *D. Q. Ma[1], I. Konidari[1], J. Jaworski[1], D. Salyakina[1], P. Whitehead[1], S. Slifer[1], J. Hoffman[1], A. Andersen[1], H. H. Wright[2], R. K. Abramson[2], J. L. Haines[3], M. L. Cuccaro[1], J. R. Gilbert[1], M. A. Pericak-Vance[1]* 1) Miami Institute for Human Genomics, University of Miami, Miami, FL; 2) University of South Carolina, Columbia, SC; 3) Center for Human Genetic Research, Vanderbilt University, Nashville, TN.

Autism is one of the most heritable neuropsychiatric disorders. No single major gene has been identified conclusively suggesting a complex genetic etiology. We performed a GWAS to help dissect its genetic complexity. 1497 samples from 488 nuclear families in our Collaborative Autism Project (CAP) database were genotyped using the Illumina Human 1M beadchip. All genotypes were called in Beadstudio and the Pedigree Disequilibrium Test (PDT) was used for association analysis. 480 trios (passing mendelian error checking) and 773,149 SNPs (Quality Control (QC): call frequency > 95% MAF > 0.05; HWE > 0.00001) were included in the analysis. For validation, we examined the AGRE 550K SNP dataset. Using the same QC thresholds, 3304 samples from 630 autism families remained in the final AGRE dataset. 74 SNPs scattered across the genome showed strong association with autism risk (PDT-P < 0.0001) in the CAP dataset, but none reached genome-wide significance. 6 of the 8 most significant SNPs were found within genes and several of these genes are expressed in brain. Five SNPs are close to previous reported candidate gene regions (2q37.1, 3p26.2, 6q15, 11p15.3, 20q13.13). Our most significant SNP is located in a novel region on chromosome 5 (25,900kb-26,100kb) in an LD block where 38 out of 52 genotyped SNPs had p-values < 0.05 and the most significant SNPs are all located in a highly evolutionarily conserved region. Among the 34 genotyped SNPs in the 550K AGRE dataset within this region, 9 replicated our result (p < 0.05) including rs4701259 and rs1428665. Analysis of copy number variants (CNVs) is underway. These data further suggest that the genetic etiology of autism is complex and that no single polymorphism carries a large effect. The replicated result on 5p14.1 strongly implicates this novel region in autism risk.

Genome-wide association scan for serum bilirubin levels in a Sardinian cohort. *M. Uda[1], S. Sanna[1], F. Busonero[1], M. G. Piras[1], G. Usala[1], A. Maschio[1], A. Mulas[1], M. Dei[1], S. Lai[1], N. Sestu[1], S. Naitza[1], L. Crisponi[1], M. Masala[1], G. Cuccuru[1], M. Marongiu[1], L. Perseu[1], R. Galanello[2], G. R. Abecasis[3], S. Schlessinger[4], A. Cao[1]* 1) INN, CNR, Monserrato, Cagliari, Italy; 2) Clinica Pediatrica, Ospedale Microcitemico, Università degli Studi di Cagliari, Italy; 3) Center for Statistical Genetics, Department of Biostatistics, University of Michigan, MI, USA; 4) Gereontology Research Center, NIA, Baltimore, MD, USA.

Bilirubin is the main bile pigment formed from the breakdown of heme in red blood cells. Serum bilirubin is considered a reliable test of liver function, as it reflects the liver's ability to take up, process, and secrete bilirubin into the bile. Within the SardiNIA population study we conducted a genome-wide association analysis in 6,148 Sardinians to identify genetic factors affecting a series of quantitative traits, including bilirubin levels. Analysis was performed in 4,305 individuals genotyped with either 10K or 500K Affymetrix GeneChip arrays. Prior to the analysis, to avoid inflation of type I error, the trait was normalized using quantile transformation. We confirmed the association of bilirubin levels with two known loci, the *UGT1A* gene ($p=6.2*10^{-62}$), and the *G6PD* gene ($p=2.5*10^{-8}$). Mutations in the *UGT1A* gene are responsible for the Crigler-Najjar syndromes as well as the more common mild hyperbilirubinemia of Gilbert syndrome, whereas deficiency in G6PD is correlated with a shorter life span of red blood cells, resulting in a modest increase in bilirubin levels. We also identified a novel association on chromosome 12 ($p=4.1*10^{-9}$). Preliminary analysis in an independent cohort of 1,862 Sardinians confirmed the association with the same direction of the effect, leading to a combined p-value of ($p=9.8*10^{-12}$). Our results suggest that other genes may be important for the regulation of serum bilirubin levels, and thus for the etiology of bilirubin- related disorders that are only partially explained by the known gene variants.

A genome-wide association study of body mass index (BMI) in African-origin samples. *S. J. Kang[1], B. Tayo[2], C. Chiang[3], T. Feng[1], A. Luke[2], R. Cooper[2], J. Hirschhorn[3,4], X. Zhu[1], H. Lyon[3]* 1) Case Western Reserve Univ, Cleveland, OH; 2) University Chicago Stritch School of Medicine, Maywood, IL; 3) Childrens Hospital Boston and Harvard Medical School, MA; 4) Broad Institute of Harvard and MIT, Cambridge, MA.

Obesity is a serious health problem that is associated with an increased risk of several common diseases. To identify genetic variants associated with obesity, we performed a genome-wide association study (GWAS) with an obesity related trait (BMI) using the Affymetrix 6.0 platform in 743 African Americans and 909 Nigerians. After quality control to remove individuals and SNPs with low quality data, we performed linear regression analysis using the residual obtained by regressing log(BMI) on age in each gender. In African-Americans, we observed 95 SNPs with P 1e-4 after correcting for population stratification (86 expected) and one SNP with p 5e-7 (0.4 expected). Among the 95 SNPs, 9 SNPs have P 0.05 in Nigerians (4.8 expected). Similarly, in the Nigerian GWAS, 112 SNPs had P 1e-4 (79 expected) and one SNP had p 5e-7 (0.4 expected). Among the 112 SNPs, 5 SNPs have P 0.05 in African-Americans (5.6 expected). To increase power, we pooled the data from African-American and Nigerian samples, correcting for population stratification. We observed 122 SNPs with P 1e-4 (79 expected), with the most significant SNP rs472039 (in a gene desert on chromosome 8; p = 3.27e-7). The SNP rs17700633 near MC4R previously found to be associated with BMI (Loos et.al. Nat. Gen., 2008) was also associated with BMI in African-Americans (p = 0.02), but not in Nigerians (p = 0.32). A pooled association test indicated a possible association (p = 0.03). Our genome-wide analysis did not yield any conclusive associations, indicating that there are not any variants of that are represented on the Affymetrix 6.0 and have large enough effects on BMI in African-ancestry populations. Our sample size may be too small to detect associations reaching genome-wide significance. However, among the list of SNPs with suggestive P values, there are likely to be valid associations that will only be detected by replication in further samples.

Pilot Newborn Screening Study for Pompe Disease in Taiwan involving 132,538 infants. Confirmation of 4 at-risk infants. *P. Labrousse[1], Y. Chein[3], R. Pomponio[2], J. Keutzer[1], N. Lee[3], S. Mann[1], C. Donohoe[1], B. Hendrickson[1], V. Akmaev[1], W. Hwu[3], T. Scholl[1]* 1) Dept Research & Development, Genzyme Genetics, Westborough, MA; 2) Molecular Genetic Analysis Group, Genzyme, Framingham, MA; 3) 2Department of Pediatrics, and Medical Genetics, National Taiwan University Hospital and National Taiwan University School of Medicine, Taipei, Taiwan.

Pompe disease is an autosomal recessive lysosomal storage disorder caused by a deficiency of lysosomal acid -glucosidase. We conducted a large Newborn Screening (NBS) pilot study in which dried blood spots from 132,538 newborns were screened for enzyme deficiency. Of the 121 newborns recalled for further testing due to reduced enzyme activity, 101 were sequenced to determine their genotypes and haplotypes. The majority of the subjects (65/101, 64.4%) were carriers of either a known deleterious mutation (38/101) or contained sequence variants of unknown significance (26/101). Ninety-nine infants carried at least one copy of the [p.G576S + p.E689K] pseudodeficient allele (GAA*03 Hap), and 35 (34.6%) were found to be homozygous for this allele only, providing evidence that this haplotype is a pseudodeficient allele. Pompe disease was confirmed by genotyping in 4 of the newborns. Overall, 15 novel variants and 6 distinct haplotypes, along with the allelic frequencies, were identified in this population. Additionally, we have characterized one new haplotype and found the frequencies to be different from those found in Caucasian and European studies. Homozygosity for the pseudodeficient allele appears to result in enzyme activity below the normal cut off for this assay in this population in the absence of any other mutations. This study shows that the NBS assay is effective in identifying infants with low GAA activity.

Genotype/Phenotype correlation in patients with Crisponi and Cold-Induced Sweating syndrome. *L. Crisponi[1], A. Meloni[1], M. Marongiu[1], F. Chiappe[1,2], M. Deiana[1], G. Zampino[3], I. Okur[4], S. Danda[5], C. Roche Herrero[6], G. Crisponi[7], F. Rutsch[8]* 1) Istituto di Neurogenetica e Neurofarmacologia, Consiglio Nazionale delle Ricerche, Cagliari, Italy; 2) University of Cagliari, Italy; 3) Departments of Paediatrics, Catholic University, Rome, Italy; 4) Gazi University Medical School, Ankara, Turkey; 5) Clinical Genetics Unit, Christian Medical College, Vellore, India; 6) Hospital Infantil La Paz, Madrid, Spain; 7) Casa di cura Sant Anna, Cagliari, Italy; 8) Department of General Pediatrics, University Childrens Hospital, Muenster, Germany.

Crisponi syndrome (CS) is a severe autosomal recessive disorder manifesting in infancy, characterized by contractions of facial muscles, dysmorphic features, camptodactyly, feeding and respiratory difficulties. Characteristic hyperthermic crisis frequently lead to death within the first months of life. Surviving patients usually develop a severe progressive kyphoscoliosis along with a paradoxical sweating after exposure to low ambient temperature. We found that mutations in the CRLF1 gene are associated with CS, showing allelism with Cold Induced Sweating syndrome type 1 (CISS1). CS and CISS1 present overlapping phenotypes, but the reason for phenotypic variation is unknown. To elucidate a genotype/phenotype correlation for both CS and CISS1, we performed functional studies on CRLF1 constructs reproducing 4 mutations found in CS and 5 mutations found in CISS1. Transfection of these mutated constructs in COS-1 cell lines revealed that all the CS mutations lead to a loss of secretion of the mutated protein; on the other hand, 3 of the 5 CISS1 mutations did not affect the secretion of the protein. Reviewing the published data on the CISS1 patients carrying the two mutations affecting secretion, we found that these patients developed a more severe phenotype than other CISS1 patients. We propose to classify the patients with CS and CISS1 as CRLF1 secreters versus non-secreters based on the effect of the mutation, and this might reflect the severity of the phenotype The continuing expansion of the mutational spectrum of CRLF1 on more cases will help us to better define this genotype/phenotype correlation.

The Coriell Personalized Medicine Collaborative- a Model for the Ethical, Legal and Responsible Implementation of Genome-Informed Medicine. *M. Christman* Coriell Institute for Medical Research, Camden, NJ.

The Coriell Personalized Medicine Collaboratives (CPMC) goal is to better understand the coming impact of genome-informed medical practice and guide its ethical, legal and responsible implementation. Coriell has formed close partnerships with several hospital partners including Cooper University Hospital, Virtua Health and Fox Chase Cancer Center - to enroll volunteers in a study that will correlate individual genome profiles of 1 million SNPs with disease course, treatment outcomes and drug responses. There is no charge to study participants. Participants control access to their genetic profiles through a secure web portal and will be able to determine whether they wish the information to become part of their medical records in the future. Participants who wish to will be able to view medically relevant information about their genomic profiles through a secure web-browser-based system. Educational material on genomics and medicine is also provided through streaming video, downloads and seminars. One goal of the CPMC is to facilitate the education of medical professionals regarding genome-derived information. To this end, many medical professionals from the hospital partners have enrolled in the study. The project has enrolled over 3,000 participants to date and will enroll ~10,000 participants in the first two to three years through the Coriell Institute and its hospital partners. There are two arms to the study: i) cancer, primarily breast and prostate cancer patients and ii) wellness, apparently healthy individuals. Only genetic variants that are deemed potentially medically actionable will be made available to participants. An Informed Cohort Oversight Board of outside scientists, physicians and ethicists will decide which risk variants will be revealed through a review of material submitted by scientists. The board (modeled after Kohane et al. (2007) Science 316, 836-837) will meet bi-anually to reevaluate and update the list of medically actionable variants. Participants may then choose to view their status for these new variants. Genetic counseling is provided at no cost to participants. Genome-informed medicine will be a national reality soon and the complex issues associated with its adoption must be addressed.

Genomewide linkage scan for split-hand/foot malformation with long-bone deficiency in a large family identifies two novel susceptibility loci on chromosome 4q21 and 9q21.2-q22.32. *M. Naveed[1], M. T. Al-Ali[1], N. Al-Khaja[1], U. Ratnamala[2], A. K. Maiti[3], C. Sun[4], M. Gains[4], K. Holden[5], D. Everman[5], CE. Schwartz[5], SE. Antonarakis[6], S. K. Nath[4], U. Radhakrishna[1,2,6]* 1) Center for Arab Genomic studies (CAGS), Dubai, United Arab Emirates; 2) Green Cross Blood Bank & Genetic Res Centre, Ahmedabad, India; 3) Sealy Center for Molecular Medicine, Univ of Texas Med Branch, Galveston, TX; 4) Arthritis and Immunology Res Program, Oklahoma Med Res Foundation, Oklahoma City, OK; 5) Center for Molecular Studies, J.C. Self Res Institute, Greenwood Genetic Center, Greenwood, SC USA; 6) Dept. of Genetic Medicine and Devp. Univ. of Geneva Medical School, Geneva, Switzerland.

Split-hand/foot malformation with long bone deficiency (SHFLD) is a rare severe limb deformity characterized by tibia abnormality with or without split-hand/split-foot deformity. Families with SHFLD have been reported with autosomal dominant and recessive inheritance, however sporadic cases exist. We recently reported large UAE family that gave significant evidence of linkage to 1q42.2-q43 and 6q14.1 (Am J Hum Genet. 80:105-11, 2007). We have recently analyzed another large multigenerational white SHFLD family (UR080) with autosomal dominant mode of inheritance and reduced penetrance. The family consists of 38 individuals including 8 affecteds (5 males and 3 females). Gnome-wide linkage analysis done on 15 members (7 affecteds) using the GeneChip Mapping EA 10K Array (Affymetrix) identified two novel loci on 4q21 and 9q21.2-q22.32. Multipoint non-parametric linkage yielded a significant evidence of linkage for several SNPs at 4q21 (NPL= 6.41, P= 0.003) and at 9q21.2-q22.32 (NPL=6.61, P=0.00098), suggests linkage at these regions. Subsequent parametric analysis also yielded a LOD score of 2.0 for each locus under an autosomal dominant with reduced penetrance model. Haplotype analysis with informative crossovers enabled the mapping of the SHFLD locus to a region of ~21.0Mb and ~26.3Mb on 4q21 and 9q21.2-q22.32 respectively. These results suggest the existence of genetic heterogeneity and suggestive evidence of two potential susceptibility loci at 4q21 and 9q21.2-q22.32 for SHFLD.

Genome-wide association analyses of composite variables identify genetic loci with pleiotropic effects on Metabolic Syndrome-related traits in GEMS Study. *H. Ling[1], D. M. Waterworth[2], K. Song[2], V. E. Moose[2], B. D. Mitchell[3], GEMS. Investigators[4]* 1) Dept Epidemiology, Univ Maryland Baltimore, MD; 2) Genetic Division, GlaxoSmithKline, King of Prussia, PA; 3) Department of Medicine, Univ Maryland Baltimore, MD; 4) GEMS Investigators.

Metabolic Syndrome (MetS) is characterized by clustering of CVD and T2DM-related risk factors, which co-occur more frequently than expected by chance. To identify genes having pleiotropic effects across multiple traits, we combined phenotype information across a set of MetS-related traits into composite variables using two different dimension-reduction approaches, a principal component analysis (PCA) and a variable cluster analysis (VCA), and then performed GWA analysis on the two sets of composite scores. Data for this study came from 1,800 subjects enrolled in the Genetics Epidemiology of Metabolic Syndrome (GEMS) Study, in whom 13 different MetS-related traits were measured. Approximate 475,000 SNPs were included after QC. Both the PCA and VCA generated a four-factor structure as their optimal solutions. Following GWA of composite traits from VCA produced no associations exceeding a genome-wide significance ($p < 1.25 \times 10^{-7}$). In contrast, 3 SNPs were significantly associated ($p < 1.25 \times 10^{-7}$) with the composite scores of PCA. These 3 SNPs were located within genes (*APOA5-APOA4-APOC3* cluster & *PSRC1*) that are widely regarded as strong candidates for CVD. Overall, the associations with the individual traits tended to be a little stronger than the associations with composite scores, although peak associations for PCA were stronger than those for VCA. Combining information across correlated traits is a commonly used data reduction approach. However, generating composite MetS-related traits from the GEMS Study using PCA and VCA did not lead to stronger associations being observed in the GWA compared to analysis of the individual constituent traits. In these data, the strongest SNP associations were observed in PCs, with SNPs in the *APOA5-APOA4-APOC3* gene cluster being associated with TG, HDL and LDLsize. SNPs in *MYBPC1* and *AGT* were associated with BP while SNPs in *TRIB1* and *PSRC1* were associated with lipids.

Distal gene expression associations in human populations. *S. B. Montgomery, B. E. Stranger, C. Ingle, C. Beazley, E. T. Dermitzakis* Population and Comparative Genomics, Wellcome Trust Sanger Institute, Cambridge, United Kingdom.

Gene expression is the product of both proximal and distal protein-DNA and protein-protein interactions. We have compared the genomic organization and relative effect of these types of interactions by quantifying gene expression and performing subsequent genome-wide association analyses in individuals derived from lymphoblast cells of the HapMap3 project (830 unrelated individuals; 1.6 million SNPs). We have identified relationships between proximal and distal associations by categorizing pathway-based interactions at different levels of association strength. Furthermore, we have hypothesized that evidence of nuclear organization should be present in genome-wide association studies of gene expression data as variation in spatially proximal regions may confer properties influencing neighbouring gene regulatory dynamics. To assess this, we have investigated the distribution of distal-associations between individual chromosomes and we have identified a unique signature of abundance of distal associations for each chromosome. We have further performed a Monte-Carlo analysis of the distribution of reciprocal distal associations and identified a window size and strength of association that is strongly overrepresented in reciprocal distal associations suggestive of direct interchromosomal interactions. We will also present analyses of distal associations within chromosomes that may be indicative of chromosome architecture within a chromosome territory, the effects of gene expression variation of CTCF, a known mediator of long-range interactions. These analyses provide evidence for distal interactions driven both by pathway effects as well as direct interchromosomal interactions. We have further dissected the distal interactions by various biological pathways and processes. This analysis has provided us with a biological framework to dissect complex biological interactions within the cell and allows for dissection of higher order effects of genetic variation.

Development of a new predictive algorithm for drug response. *S. Lupoli[1,2], F. Torri[2], L. Citterio[3], C. Barlassina[2], A. Orro[4], F. Martinelli Boneschi[1], L. Milanesi[4], D. Cusi[5,6], G. Bianchi[3], F. Macciardi[2]* 1) INSPE, San Raffaele Scientific Institute, Milan, Italy; 2) University of Milan, Milan, Italy; 3) San Raffaele Hospital, Milan, Italy; 4) ITB, CNR, Milan, Italy; 5) San Carlo Borromeo Hospital, Milan, Italy; 6) GENOPOLIS, University of Milan, Milan, Italy.

Pharmacogenetics (PGx) i.e the use of genetic analysis to predict drug response, efficacy and toxicity, is becoming the first pipeline technology to address new drug discovery. There is strong evidence that both genetic heterogeneity and genetic complexity are to be expected in drug response. Therefore, much attention has been placed on association / Linkage Disequilibrium methods, even across the entire human genome, since they can deal more appropriately with the issues of complexities. Technically, the recent improvement in the technology of high-throughput SNP genotyping and the availability of a large number of SNP databases make association studies and fine mapping of disease loci more convenient using a Genome-wide Association Study (GWAS) framework. The next critical issue relates to the definition of the phenotype and to the use of bioinformatics to build a predictive model based on the genetic profile. We performed a GWAS and we identified the genes that control for the drug effect. We adopted a quantitative genetic association design, where the factors affecting the distribution of the phenotype are the SNPs, the therapy group and the SNP*therapy interaction. Thus, for each SNP that we investigate, the following linear model is built: Phenotype = SNP + therapy + SNP*therapy. Once we identified the genes that control for the drug effect, we developed an algorithm to detect the genotypic profiles that discriminate responders from non-responders using the minimal set of significant SNPs. It is in fact highly desirable both clinically and economically to establish models to distinguish responders from non-responders and to predict possible outcomes of treatment. We present an example of this approach, with an algorithm that has the ability to predict outcomes when the relationships between the variables are multidimensional and nonlinear as found in complex medical applications.

The Kinesin Family Member 6 Trp719Arg variant does not associate with coronary artery disease in the Ottawa Heart Genomics Study. *A. F. R. Stewart, S. Dandona, O. Assogba, M. Belanger, L. Chen, G. A. Wells, R. McPherson, R. Roberts* Univ Ottawa Heart Inst, Ottawa, ON, Canada.

Background: Several recent reports have identified an association between the 719Arg variant of KIF6 and incident coronary events. In some instances, this association has been extended to include coronary artery disease (CAD). **Methods**: Here, we tested whether the KIF6 719Arg variant is associated with CAD in early onset cases (average age 49 years) defined by coronary angiography as having greater than 50% stenosis of any coronary artery versus asymptomatic elderly (average age 75 years) controls. 1540 cases (45.8% with myocardial infarction) and 1455 controls were genotyped on the Affymetrix 500K or 6.0 SNP microarrays. High resolution melting curve genotyping of rs20455 was also carried out in 957 cases and 1004 controls. **Results**: KIF6 Trp719Arg variant showed no association with coronary artery disease (p=0.523 for trend test). Moreover, among 706 cases with myocardial infarction, no association with coronary events was detected when compared to 756 non-MI CAD cases (p=0.588). Concordance between Affymetrix array and high resolution melting curve genotyping data was 98%, confirming the accuracy of genotypes. Data from the Wellcome Trust Case Control Consortium (WTCCC) also showed no significant association with CAD (p=0.897). **Conclusion**: The KIF6 Trp719Arg variant does not associate with myocardial infarction or with coronary artery disease in the Ottawa Heart Genomics Study.

A Genome Wide Linkage Study of Endophenotypes for Schizophrenia in Extended Families. *M. F. Aukes[1], B. Z. Alizadeh[2], M. M. Sitskoorn[1,3], C. Kemner[1], R. van 't Slot[2], B. P. C. Koeleman[2], R. A. Ophoff[2,4], R. S. Kahn[1]* 1) Rudolf Magnus Institute of Neuroscience, Psychiatry Dept, UMC, Utrecht, Netherlands; 2) Complex Genetics Section, Medical Genetics Dept, UMC, Utrecht, Netherlands; 3) CoRPS, Tilburg University, Tilburg, Netherlands; 4) UCLA Center for Neurobehavioral Genetics, Semel Institute for Neuroscience and Human Behavior, Los Angeles, CA.

Genetic studies of schizophrenia have not resulted so far in the detection of causal variants for the disorder. Linkage analysis of endophenotypes for schizophrenia may aid in finding loci that contain genes affecting the disorder. Both the use of quantitative traits and extended families can improve power in a linkage design. First we performed heritability, segregation and bivariate heritability analyses to select six promising endophenotypes for schizophrenia: sensorimotor gating, openness, verbal fluency, early visual perception, spatial working memory, and IQ. Then, we performed a genome-wide high-density linkage scan in 7 extended multiple affected Dutch pedigrees (n=125, including 174 parent-offspring pairs, 141 sibling pairs, 110 grandparent-grandchild pairs, and 207 avuncular pairs), using 6090 single nucleotide polymorphism markers (SNPs) (Illumina Infinium Humanlinkage-12), with a mean interval of 0.441 Mb. We removed SNPs showing ambiguous clustering (n=80), and checked for Mendelian inconsistencies (using Pedcheck, n=2), allelic frequencies and gender errors (using PLINK, n=0). Variance-component based linkage analysis was performed in SOLAR and MERLIN. Our preliminary results show suggestive linkage (LOD1.7) for openness on 2p24 (LOD=2.02) and 2q31 (2.17), sensorimotor gating on 5q33 (2.3), spatial working memory on 2q33 (1.71) and 15q13 (2.15), and for IQ on 12p13 (2.29). Peaks overlapped among traits on 2p24, 2q31-33, 3p22-21, 11q14-22, 12p13, 15q13 and 17q25. Moreover, several peaks were detected in regions associated in previous linkage studies with schizophrenia and/or endophenotypes (e.g. 5q33, 3p22-21, 15q13). This study provides the first genome wide scan for several candidate endophenotypes for schizophrenia. This approach may thus enhance the detection of loci associated with schizophrenia.

Role of *PRKRA* in the development of isolated microtia. *J. S. Guimbellot[1], L. C. Pyle[1], E. G. Spencer[1], D. Hurtado[2], K. Johnson[1], T. Callens[1], L. O. Vasconez[3], H. C. Vasconez[3], B. Engels[3], R. J. Fix[3], N. H. Robin[1], L. Messiaen[1]* 1) Dept Genetics, UAB, Birmingham, AL; 2) Pontificia Universidad Católica, Quito, Ecuador; 3) Dept Surgery, UAB, Birmingham, AL.

Microtia is a developmental abnormality of the ear, resulting in significant malformation of the middle and outer ear.Recent studies have suggested a link between several loci and microtia, including mutations in *FGF3*, *HOXA2*, and a copy-number variation (CNV) at chromosome 4p16.In addition to microtia, other anomalies were also found in these patients.Another candidate gene was suggested by a mouse model with a non-functional *PRKRA*, which has a similar phenotype to that of humans with isolated microtia.We hypothesized that mutations in the *PRKRA* gene may play a role in the development of isolated microtia.We collected 52 saliva samples from microtia patients, non-affected family members, and unrelated controls in Ecuador, where a high incidence of microtia is found.The study was approved by the UAB IRB and the Ecuador Ministry of Health.We conducted a thorough history and physical assessment of congenital anomalies and asymmetry.DNA was extracted from all samples using a salting-out method, followed by amplification and sequencing of all eight exons of *PRKRA* and the 5UTR.

Our results revealed no association with any historical factors.Asymmetry of the ears, maxilla and mandible, but of no other features, was found in the case group and not the control group.The most common variant, c.236-130_236-128dupGTA, was found in both groups and is likely a normal variant among this population.No clearly pathogenic mutations that could account for the development of microtia were found.While our study is limited by the number of enrolled individuals, it suggests mutations in the coding region of *PRKRA* are not responsible for the development of isolated microtia as seen in the mouse model, although we did not assess the presence of dosage alterations.It will be of interest to evaluate our patient population for mutations in *FGF3*, *HOXA2*, and the CNV on 4p16 to assess whether these loci play a role in the development of microtia in the absence of other anomalies.

Clinical effects of nonadherent cells on children with osteogenesis imperfecta. *R. Jethva[1], M. Dominici[2], P. Gordon[3], T. J. Hofmann[1], E. M. Horwitz[1,4]* 1) The Children's Hospital of Philadelphia, Philadelphia, PA; 2) University of Modena and Reggio Emilia, Modena, Reggio Emilia, Italy; 3) Penn State Milton S. Hershey Medical Center, Hershey, PA; 4) The University of Pennsylvania, Philadelphia, PA.

Bone marrow transplantation (BMT) is an effective therapeutic modality for many genetic and acquired diseases of the hematopoietic system. Recently, it has been shown that hematopoietic cells in the marrow are capable of both blood and bone cell differentiation. Allogeneic BMT in children with severe osteogenesis imperfecta (OI) resulted in engraftment of donor-derived cells with both hematopoietic and osteopoietic differentiation. Clinical outcomes included improvements in growth, bone histomorphometry, fracture rate and bone mineral content. Subsequent adherent mesenchymal stromal cell (MSC) transplantations were performed in the same OI patients, which also resulted in improvements in growth. Murine studies with nonadherent cells (NAC) have shown that NACs are capable of significantly greater engraftment in bone than MSCs and are likely to harbor stem cells capable of dual potential. To investigate their clinical impact, NACs were harvested from the donors of the original studies. Cells were CD3-depleted and had low MSC counts prior to transplantation. In two 3-month periods following transplant, growth was documented. Patients 1 and 2 were considered non-responders with no more than 1cm growth. Patients 3, 4 and 5 were considered responders, with a maximum growth of 3.3cm. No correlation was found between responders and the absolute number of MSCs or CD34+ cells transplanted (Pearsons test). It was noted that the same two patients who were non-responders had responded the least in the prior allogeneic BMT trial whereas there was no difference in their growth patterns compared to other patients during the MSC trials. Each child had a different mutation, suggesting the possibility that the effects of NACs and MSCs may work based on mutation-dependent and independent mechanisms, respectively. Further study is necessary to characterize these possible associations, which could have implications on cell therapy for OI.

Using principal components analysis to identify candidate genes for natural selection. *P. Paschou[1], J. Lewis[2], P. Drineas[2]* 1) Dept of Molecular Biology & Genetics, Democritus University of Thrace, Greece; 2) Dept of Computer Science, RPI, USA.

Genetic markers that differentiate populations are excellent candidates for natural selection due to local adaptation, and may shed light into physiological pathways that underlie disorders with varying frequencies around the world. Principal Components Analysis (PCA) has emerged as a powerful tool for the characterization and analysis of the structure of genomewide datasets. In prior work, we described an algorithm that can be used to select small subsets of genetic markers (SNPs) that correlate well with population structure, as captured by PCA. Our method can be used to detect SNPs that differentiate individuals from different geographic regions, or even neighboring subpopulations. We set out to explore the nature and properties of the genes where population-differentiating SNPs reside, by analyzing the publicly available Human Genome Diversity Panel dataset (650,000 SNPs for 1,043 individuals, 51 populations). Applying our SNP selection algorithms, we chose small subsets of SNPs that almost perfectly reproduce worldwide population structure as identified by PCA. We determined SNP panels both for population differentiation within seven geographic regions, as well as around the globe. We then explored the hypothesis that the selected SNPs attained their current worldwide allele frequency patterns as a response to the pressure of natural selection. Comparing our lists to recently published reports, we found a significant overlap with other genomewide scans for selection, thus validating our hypothesis. For example, EDAR (involved in the development of hair follicles) harbors the most differentiating SNPs in our world-wide panels. SNPs located in genes that are involved in skin and eye pigmentation (OCA2, MYO5C, HERC1, HERC2) are also among the top population differentiating markers. In East Asia, SNPs residing at the ADH cluster appear among the most important SNPs for population structure, while, in Europe, the same is true for genes that are involved in immune response to pathogens (CR1, DUOX2, TLR, and HLA). Finally, a comprehensive gene ontology analysis is presented.

GENOME-WIDE PROFILES OF SNP AND COPY NUMBER VARIATION IN 1,260 HAPMAP INDIVIDUALS FROM MULTIPLE GLOBAL POPULATIONS. *E. Dermitzakis*[1], . *BI*[2], . *BCM*[3], . *WTSI*[1] 1) Wellcome Trust Sanger Inst, Cambridge, United Kingdom; 2) Broad Institute of Harvard and MIT, Boston, USA; 3) Baylor College of Medicine, Texas, USA.

Efforts to understand the genetic basis of disease increasingly analyze copy-number variation and SNPs, in large diverse sample sets. The International HapMap Consortium described an initial map of 3.1 million SNPs in 270 individuals from 4 population samples, enabling a first generation of genome-wide association studies. To enable next-generation investigations and to better understand patterns of global human genetic variation, we set out to make an even, dense map of SNPs and CNVs across 1,260 individuals from 11 population samples, (African Ancestry in SW USA; Chinese in Metropolitan Denver, CO, USA; Gujarati Indians in Houston, TX, USA; Luhya in Webuye, Kenya; Maasai in Kinyawa, Kenya; Mexican Ancestry in LA, CA, USA; Toscani in Italy; and additional samples from the 4 populations sampled in HapMap. Both the Affymetrix SNP 6.0 and the Illumina Infinium 1M SNP Arrays were used to measure SNP and CNVs in these individuals. In addition, 10 genomic regions (total size 1 Mbp) were sequenced in more than 800 samples. Using data from both platforms, we constructed a relatively even and dense SNP map and a high-quality CNV map, providing estimates of allele frequencies in different populations, and distinguishing among rare, common, and de novo CNVs. We will present comparison of the newly generated data with previous datasets, analysis of linkage disequilibrium, haplotype structure, and population differentiation across these 1,260 samples and how the estimates compare to those inferred from smaller sample sizes. We will also present evaluations of SNP ascertainment and coverage through imputations for both common and rare variants. Based on combined SNP and CNV data we will present the genomic and population-genetic properties of CNVs and tagging provided by SNPs in these population samples. Finally, we will describe genome-wide inference of recent natural selection. These public datasets provide a high-resolution framework for genome-wide association studies and populations not previously represented in HapMap, and integrate SNP and copy number variation with high accuracy.

Association analysis of 42 hereditary prostate cancer (HPC) families using segregating risk haplotypes identifies a 20Kb region on chromosome 22q12.3. *B. Johanneson[1], SK. McDonnell[2], JL. Stanford[3], DM. Karyadi[1], DJ. Schaid[2], L. Wang[2], K. Deutsch[4], L. McIntosh[3], JR. Cerhan[2], JL. St. Sauver[2], SN. Thibodeau[2], EA. Ostrander[1]* 1) NHGRI, National Institutes of Health, Bethesda, MD; 2) Department of Health Sciences, Mayo Clinic, Rochester, MN; 3) Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA; 4) Institute for Systems Biology, Seattle, WA.

Multiple studies of HPC families identify a susceptibility locus at chromosome 22q12. In this study, a set of 24 families from the Mayo Clinic and 18 from the Seattle-based PROGRESS study, each with family-based LOD scores 0.58 at 22q12 were initially used for fine mapping and mutation scanning. Fourteen families with 5 affected men highlight a 2.53 Mb minimal recombination interval. A set of 202 SNPs were genotyped in the 18 PROGRESS families. Markers were selected from among 670 SNPs previously genotyped in 498 Mayo family-based cases and 533 population-based controls which yielded a p-value 0.05 in a family-based association study. Fifty-two of these SNPs are located inside the 2.53 Mb recombination interval described above. We then used the pedGenie software to test all 202 SNPs in the 18 PROGRESS families and the 24 Mayo families as well as the 533 population-based controls. Monte Carlo simulation compensated for related individuals. By assessing haplotype sharing between affected family members, unassigned individuals were defined as likely carriers of the at risk haplotype or not. This allowed us to exclude potential phenocopies and healthy carriers, increasing the power and accuracy of the overall data set. Preliminary results highlight a 20 Kb region within the 2.53 Kb minimal recombinant interval indicated by 3 SNPs that includes 1 or 2 candidate genes. The association is observed independently in both the Mayo and PROGRESS data sets, as well as the combined set of 42 families. We are currently sequencing within and surrounding the candidate gene/s to identify putative causative SNPs which will be assessed in a larger population-based, case control study of 2800 individuals.

Multi-population HLA allele prediction from SNP data. *S. Leslie[1], L. Ohm-Laursen[2], R. Bowden[1], J. Frater[2], D. Goedhals[3], C. van Vuuren[3], C. Seebregts[4], P. Donnelly[1], R. Phillips[2], G. McVean[1]* 1) Department of Statistics, University of Oxford, 1 South Parks Road Oxford, OX1 3TG, UK; 2) The Peter Medawar Building for Pathogen Research, University of Oxford; 3) University of Free State, Bloemfontein, South Africa; 4) Medical Research Council (South Africa).

Genetic variation at classical HLA alleles is a crucial determinant of transplant success and susceptibility to a large number of infectious and autoimmune diseases. Large-scale studies involving classical type I and type II HLA alleles are currently limited by the cost of allele typing technologies. Recently we developed a statistical method using SNP variation within the MHC region to predict alleles at key class I (HLA-A, HLA-B and HLA-C) and class II (HLA-DRB1, HLA-DQA1, HLA-DQB1) loci (Leslie et al. 2008. Am. J. Hum. Genet. 82: 48 - 56). This uses a population genetic approach, combined with a database of individuals with known HLA and SNP alleles within the MHC region, to predict HLA alleles for individuals for which only SNP information is known. The method promises to facilitate large-scale experiments, including disease-association studies and vaccine trials, where detailed information about HLA type is valuable. We present two further studies of the efficacy of this method. Firstly we study the effect of increasing the database size on the accuracy of predictions and report results for populations of European ancestry. Secondly, we present the results of a pilot study testing the efficacy of the method in samples of African origin. It is known that there is a high degree of human genetic variation in Africa and that there are considerable differences between populations of different origin. To study the effect of these factors on the HLA allele prediction method we obtained approximately 350 samples from South Africa, determined their HLA and SNP allelic types, and compared the accuracy of predictions using different training data sets: The HapMap Yoruba samples, subsets of the South Africa samples and samples of European origin.

c.1392+2TC mutation in MFN2 gene affects splicing in Charcot-Marie-Tooth (CMT) 2A family. *A. Vettori[1], F. Boaretto[1], G. Vazza[1], M. Muglia[2], A. Martinuzzi[3], A. Paticucci[2], C. Bertolin[1], G. Bergamin[1], A. Quattrone[2], M. L. Mostacciuolo[1]* 1) Dept. Biology, University of Padova, Padova, Italy; 2) Institute of Neurological Sciences, National Research Council, Mangone, Cosenza, Italy; 3) IRCCS E. Medea, Conegliano Research Center, Italy.

Mutations in the MFN2 gene have been reported as the primary cause of the axonal form of Charcot-Marie-Tooth disease (CMT type 2A), a peripheral neuropathy characterized by axonal degeneration. The MFN2 gene maps in the 1p36 and codes for mitofusin 2 (mfn2) protein that belongs to a class of highly conserved mitochondrial transmembrane GTPases. By a functional point of view, mfn2 is an essential component of the mitochondrial fusion machinery and thus involved in the regulation of mitochondrial dynamics. In addition, recent reports shed new light on the physiological importance of mfn2 function suggesting a role in mitochondrial metabolism, apoptosis as well as cellular signalling. Despite these knowledge gains, several issues regarding mfn2 function and its relation to the neurodegenerative disease remain poorly understood. Here we preset the genetic study in a two-generation family in which three affected members developed CMT2 symptoms in the fifth decade of life (late onset). All patients experienced a rapid worsening of clinical features and became dependent on wheelchair in only two years. Moreover, few years after the onset of symptoms all affected subjects suddenly died for encephalopathy. Conversely to the majority of mutations detected so far, that are clustered in the GTPase domain, we identified a point mutation in intron 13 affecting the conserved consensus sequence of the donor splice site (c.1392+2T>C). Lymphocytes and in vitro transcript analysis reveled that the mutated allele generates four different aberrant transcripts with partial or total intron-13 retention. If translated, these mRNA give raise to either C-truncated, shorter or longer mfn2 proteins compared to the wild type. The functional study of each one of these protein isoforms may help explain the unusual phenotype we observed in this family and contribute to elucidate the potential pathogenic mechanisms involved in CMT2A neurophaty.

Association of a common *G6PC2* variant with fasting plasma glucose levels. *F. Y. Demirci[1], A. S. Dressen[1], R. F. Hamman[2], C. M. Kammerer[1], M. I. Kamboh[1]* 1) Dept. of Human Genetics, GSPH, Univ. of Pittsburgh, Pittsburgh, PA; 2) Dept. of Preventive Medicine and Biometrics, Univ. of Colorado Denver, Aurora, CO.

Fasting plasma glucose (FPG) levels are genetically influenced (heritability estimates; 25-40%) and correlate with mortality and cardiovascular disease in both diabetic and non-diabetic subjects. *G6PC2* encodes a pancreatic islet-specific glucose-6-phosphatase-related protein and *G6pc2*-null mice were reported to exhibit a mild metabolic phenotype with decreased FPG levels. Two recent genome-wide association studies (Bouatia-Naji et al. *Science* 2008 & Chen et al. *J Clin Invest* 2008) have implicated a role for *G6PC2* in contributing to inter-individual variation in blood glucose levels in the general population. The rs560887 SNP [minor allele frequency (MAF) = 0.30] was reported to be associated with FPG levels in normoglycemic subjects but not with type 2 diabetes (T2D). This observation led to the hypothesis that the genetic determinants of FPG levels in physiological states may be different than those associated with overall T2D risk. The rs560887 SNP is located in intron 3 of *G6PC2* close to acceptor splice-site and is predicted to affect alternative RNA splicing. The purpose of this study was to replicate the association of the rs560887 SNP in our independent epidemiological sample of American non-Hispanic Whites. DNA samples from a total of 623 non-diabetic individuals (328 women and 295 men) were genotyped using the TaqMan SNP genotyping assay for rs560887. The genotype frequencies were in Hardy-Weinberg equilibrium and the allele frequencies (MAF = 0.33) were similar to those previously published or reported in public databases. The rs560887 SNP was significantly associated with FPG levels in our sample after adjusting for gender, age, and BMI (p=0.003 under the additive model). Consistent with recent reports, no association was detected with fasting insulin levels, BMI, or lipid measurements. Our results in an independent sample confirm the robust association of the *G6PC2*/rs560887 SNP with FPG levels in non-diabetic individuals and further supports the relevance of the glucose phosphorylation pathway to glucoregulation in the general Caucasian population.

Genome wide analysis and heritability estimation of intelligence in the International Multi-centre ADHD Genetics (IMAGE) study. *A. Arias-Vasquez[1,2], T. Sampaio Rizzi[3], N. Lambregts-Rommelse[1], K. Zhou[4], P. Asherson[4], E. Sonuga-Barke[5], M. Gill[6], J. Sergeant[7], R. Ebstein[8], A. Rothenberger[9], H. C. Steinhausen[10], T. Banaschewski[11], R. Oades[12], A. Miranda[13], F. Mulas[13], H. Roeyers[14], J. Buitelaar[1], D. Posthuma[3], B. Franke[2,1], S. Faraone[15] for The IMAGE Group* 1) Psychiatry, UMC St. Radboud, Nijmegen, NL; 2) Human Genetics, Nijmegen, NL; 3) Biological Psychology, Vrije Universiteit, Amsterdam, NL; 4) London, UK; 5) Southampton, UK; 6) Dublin, Ireland; 7) Amsterdam, NL; 8) Jerusalem, Israel; 9) Göttingen, Germany; 10) Zurich, Switzerland; 11) Mannheim, Germany; 12) Essen, Germany; 13) Valencia, Spain; 14) Ghent, Belgium; 15) SUNY, Upstate Medical University, Syracuse, NY, USA.

Attention-Deficit/Hyperactivity Disorder (ADHD) is a neurodevelopmental disorder characterised by symptoms of inattention, hyperactivity and impulsivity. There is growing evidence of heterogeneity in its etiology, pathophysiology and clinical expression. One approach to resolving heterogeneity involves the identification of endophenotypes, intervening variables that might mediate pathways between specific genes and clinical phenotype. IQ is a candidate endophenotype for ADHD. Genome-wide linkage analyses of full scale IQ and IQ subscales were performed in the International Multi-centre ADHD Genetics (IMAGE) study including 1094 families with 1094 DSM-IV combined type ADHD probands and their 1441 siblings (unselected for ADHD status). IQ was measured using five subscales of the WISC-IIIR scale. The full scale prorated IQ score and the five subscales were used as quantitative traits for linkage analysis. 5,407 autosomal SNPs were used to run multipoint regression-based linkage analyses using MERLIN. The h^2 estimates from the IQ subscales and the full IQ score ranged from 31% to 100%. Three suggestive linkage signals were found (LOD scores 2, p values 0.001) on chromosomes 7, 9 and 14 for three different subscales. Previously, two regions on chromosomes 7 and 14 were reported as being associated or linked to IQ. Our results, though only suggestive, suggest the presence of additional genetic variants contributing to the variance of IQ in ADHD.

Fine mapping of multiple causal MHC variants using imputation and shrinkage regression. *C. Vignal*[1,2], *A. Bansal*[2], *D. Balding*[1] 1) Imperial College, UK; 2) GlaxoSmithKline, UK.

Risk of rheumatoid arthritis (RA) is associated with the MHC region, most notably with a group of alleles termed the shared epitope (SE) at HLA-DRB1. Additional MHC loci may also play a role but inference is hampered by the presence of high linkage disequilibrium (LD) and differences in SNP coverage across studies. We investigate genotype imputation at untyped and typed markers as a means to jointly analyse correlated SNPs from multiple studies.

Genotype imputation was applied, using MACH, to data from the Wellcome Trust Case Control Consortium (WTCCC), HapMap (Caucasian parents) and 977 RA cases and 855 controls from the GoRA study, whose subjects had been genotyped for 2,302 SNPs, together with HLA-DRB1 locus. After imputation, the combined dataset comprised 6,647 subjects and 9,990 genetic markers from the MHC region. Uncertainty in imputation was taken into account in the association analyses by using the estimated minor allele count, averaged across MACH iterations. We adopted a Bayesian-inspired penalised logistic regression approach for variable selection, adjusting for the effect of the SE at HLA-DRB1. We investigated a range of prior (penalty) functions, including the Laplace (double exponential). Parameter inference was based on the posterior mode, with non-zero values indicating marker-disease associations.

Careful choice of burn-in improved the performance of MACH. After controlling for type-I error, univariate association tests identified many positive associations in the combined GoRA-WTCCC dataset, most of them likely to be spurious. Shrinkage regression better handled the correlation between predictors and greatly reduced the number of positive signals. Moreover the positive signals identified were not strongly correlated with each other or with SE. Thus imputation and shrinkage regression have identified a number of independent candidates associated with RA in the MHC region, and these are being further investigated.

Somatic events in retinoma and retinoblastoma. *M. Amenduni[1], K. Sampieri[1], F. Ariani[1], F. T. Papa[1], M. Bruttini[1], M. A. Mencarelli[1], M. C. Epistolato[2], P. Toti[2], S. Lazzi[2], A. Marozza[1], F. Mari[1], T. Hadjistilianou[3], S. De Francesco[3], A. Acquaviva[4], A. Renieri[1]* 1) Medical Genetics, Univ of Siena, Italy; 2) Human Pathology and Oncology Dept, Univ of Siena, Italy; 3) Ophtalmology Dept, Univ of Siena, Italy; 4) Pediatrics Dept, Univ of Siena, Italy.

To characterize somatic events leading from normal retina (Rt) to retinoma (Rn) and from Rn to retinoblastoma (Rb), we used 3 different approaches on two eye samples showing areas of Rn adjacent to Rb: i) RB1 mutational analysis; ii) qPCR at 4 genes involved in Rb pathogenesis (MDM4, MYCN , E2F3 and CDH11); iii) array-CGH. We demonstrated that 2 RB1 mutational hits are already present in Rn. In contrast with the previous model, qPCR showed that MYCN and E2F3 amplifications are already present in Rn tissue. The level of amplification is increased in Rb cells. Array CGH revealed interesting and different results in the two cases. In Case#1, Rn was documented by ophthalmoscopic examination as a lesion stable for 11 months before Rb transformation. In this case, no genomic rearragements are present in Rn. In Case#2, belonging to a patient diagnosed with advanced Rb, Rn tissue was observed by retrospective histopathological examination. In this case, Rn shares 3 genomic rearrangements with Rb (dup5q13.2, dup6p, dup8p23.1). Two other rearrangements without any known gene are Rn specific (dup1q32.2 and dup13q31.2). One rearrangement (dup5p) is present only in Rb and contains several candidate genes for malignant transformation, including SKP2 (p45), essential for S-phase entry of cell cycle. These results suggest that the two lesions named retinomas are different in nature. The first is a pretumoral lesion, while the second represents a clone of cells that presents benign rearrangements different from those acquired by another clone generating Rb. To characterize recurrent genomic rearrangements in Rb tissues, we analysed 18 tumor eye samples by array CGH. We detected 7 common rearrangements including dup6p (E2F3), dup2p24.1 (MYCN), dup1q (MDM4), del13q13.2-22.3 (RB1) and del16q12.1-12 (RBL2 encoding p130). Overall, these results emphasize the role of these oncogenes/oncosuppressors in Rb tumor progression.

Promoter region in the ARG1 gene is involved in the genetic risk for asthma in Puerto Rican patients. *M. Via[1], C. Eng[1], S. Choudhry[1], H. Corvol[1], M. A. Seibold[1], R. Chapela[2], J. R. Rodríguez-Santana[3], W. Rodríguez-Cintron[4], P. C. Ávila[5], E. G. Burchard[1]* 1) Dept Medicine, Univ California, San Francisco, San Francisco, CA; 2) Instituto Nacional de Enfermedades Respiratorias, Mexico City, Mexico; 3) Centro de Pneumología Pediátrica, San Juan, Puerto Rico; 4) Veteran Affairs Medical Center, San Juan, Puerto Rico; 5) Northwestern University School of Medicine, Chicago, IL.

Arginase-1 (ARG1) gene has been identified as a candidate gene for asthma in animal models. Moreover, a recent study has established an association between variants in ARG1 gene and atopy among Latino subjects with asthma. In this study we have analyzed five SNPs in the ARG1 gene region in a family-based association study of Mexican and Puerto Rican asthmatic patients (N=687 families). An association was found between allele C of SNP rs2781664 located in the promoter region and an increased risk for asthma only among Puerto Rican families (OR=1.38; 95%CI 1.08-1.77; P=0.009). In these patients, this variant was also associated with higher levels or increased risk for all recorded allergy-related phenotypes such as IgE levels (P=0.018), number of factors causing runny/stuffy nose or cough (P=0.006), eczema (P=0.033) and hay fever (P=0.023). The same results were obtained for the most common haplotype in the promoter region (C-T-T-T), and partially for other SNPs in tight linkage disequilibrium. Luciferase expression assays were performed to assess the potential functional role underlying the observed associations. Haplotype C-T-T-T showed higher levels of expression at the basal transcription level, but differences were not statistically significant. Although not conclusive, this trend towards higher transcription levels could imply a more severe allergic asthmatic response and be the causative factor for the associations found.

Identification of SNPs underlying parental preferential effects on gene expression levels. *C. A. Anderson, B. M. Herrera, M. Jain, A. P. Morris, C. M. Lindgren* Wellcome Trust Centre for Human Genetics, University of Oxford, United Kingdom.

Recently, many studies have attempted to correlate gene expression levels to genotypic variation. Such studies have followed similar paradigms to traditional association and linkage analyses. Thus far, the identification of SNPs which underlie parental preferential gene-expression effects has been neglected. The identification of such SNPs, where the effect of the inherited allele differs depending from which parent it was inherited, would reveal an as yet uncharacterised mechanism underlying human phenotypic variation. Here, we use publicly available gene-expression data obtained from the CEU HapMap samples in an attempt to identify such variants. Only expression traits with a standard deviation 0.5 were taken forward. We identified all SNPs 500kb downstream and 50kb upstream of each gene transcript (i.e. our analysis was restricted to the identification of SNPs with cis-acting effects on parental preferential gene-expression). The list of SNPs under analysis was further reduced so that no pair of SNPs had an r^2 0.99. In total, 173,527 SNPs and 1,327 gene-expression traits were analysed and 212,933 tests were performed. 4 SNPs (from 3 unique transcripts) were identified showing significant evidence (p2.3×10^{-7}) of parental preferential effects on gene expression. We attempted replication using gene-expression and genotypic data from the Yoruban HapMap individuals and saw nominal evidence of parental preferential effects (p0.05) for 1 SNP. Permutation analysis is planned to better define significance thresholds and replication will be attempted in a large independent cohort of European descent.

Confirmation of novel prostate cancer susceptibility loci in men with early-onset prostate cancer. *K. Zuhlke[1], A. Ray[1], S. Walters[1], E. Lange[3,4], K. Cooney[1,2]* 1) Dept Internal Medicine, Univ Michigan, Ann Arbor, MI; 2) Dept of Urology, Univ Michigan, Ann Arbor, MI; 3) Dept of Genetics, Univ North Carolina, Chapel Hill, NC; 4) Dept of Biostatistics, Univ North Carolina, Chapel Hill, NC.

Large genome-wide association studies (GWASs) continue to emerge as a powerful approach for identifying common disease alleles associated with complex human diseases. A recent publication by Eeles et al. (2008) performed a two-stage GWAS with familial and early-onset prostate cancer cases from the UK and Australia that confirmed the association between prostate cancer and SNPs in previously reported regions on chromosomes 8 and 17. In addition, Eeles et al. identified seven new prostate cancer susceptibility loci on chromosomes 3 (rs2660753), 6, (rs9364554), 7 (rs6465657), 10 (rs10993994), 11 (rs7931342), 19 (rs2735839), and X (rs5945619), that reached global statistical significance ($p < 2.7x10-8$). In this study, we genotyped these seven newly identified SNPs in 750 unrelated Caucasian prostate cancer cases diagnosed at or before the age of 55 (avg. age dx = 49.8 yrs 3.9) from the University of Michigan Prostate Cancer Genetics Project (PCGP) and compared their genotype frequencies to 3,085 self-reported Caucasian controls from Illuminas iControlDB database. Unconditional logistic regression models were used to evaluate the association between prostate cancer and each SNP. We found confirmatory evidence ($p < 0.05$ and the association in the same direction as in Eeles et al.) to support an association for six of the seven novel SNPs and early-onset prostate cancer. Only rs2660753 on chromosome 3 was not significantly associated with prostate cancer, despite our sample having a higher frequency of the risk allele than that reported in both Stage 1 and Stage 2 samples reported by Eeles et al. We believe that early-onset prostate cancer is an important public health problem and that studies using early-onset prostate cancer cases will have the greatest power to detect genetic variants associated with the disease.

Single nucleotide polymorphisms in the BDNF gene affect cognitive measures shortly after traumatic brain injury. *T. W. McAllister[1], A. L. Tyler[1], L. A. Flashman[1], C. H. Rhodes[1], A. J. Saykin[2], B. C. McDonald[2], T. D. Tosteson[1], G. J. Tsongalis[1], L. L. Hoskins[1], J. H. Moore[1]* 1) Dartmouth Medical School, Dartmouth College, Lebanon, NH; 2) Indiana University School of Medicine, Indianapolis, IN.

Different functional outcomes are often reported in individuals with similar degrees of traumatic brain injury (TBI) suggesting that environment and genotype play important roles in outcome. Polymorphisms in genes responding to neurotrauma or modulating cognition-associated neurotransmitter systems may influence either the acute response to trauma or adaptation to injury. Brain Derived Neurotrophic Factor (BDNF) plays a role in neural survival, repair, and plasticity, and facilitates both early and late long-term potentiation. A non-synonymous SNP (rs6265) in BDNF has been shown to affect human memory function. We hypothesized that this SNP and neighboring SNPs would affect cognitive function shortly after MTBI. 75 consecutive patients with mild-moderate TBI (MTBI) were recruited from a Level-1 trauma center emergency department, studied approximately one month after injury and compared to 38 healthy subjects. Written informed consent was obtained from all participating subjects. Participants were tested in areas of learning and reaction time and genotyped across 9 SNPs within the BDNF using a custom made 3600 SNP microarray gene chip (Affymetrix, Inc., Santa Clara, CA). Adjusting for age and education, and using an FDR of 0.1, we found a significant main effect of three on measures of processing speed (p0.005). Within the MTBI group, there were significant effects of genotype on processing speed and memory. Cognitive effects were most prominent in male participants. A six SNP haploblock in male MTBI patients was most predictive of outcome. This study provides support for the hypothesis that polymorphisms in the BDNF gene may influence cognitive performance shortly after MTBI, particularly in males. The mechanism is unclear but may be related to alterations in BDNF expression or function in the hippocampus.

Adenylosuccinate lyase (ADSL) deficiency. *M. Zikanova, V. Skopova, L. Dvorakova, J. Krijt, S. Kmoch* Charles University, 1st Faculty of Medicine, IIMD, Prague, Czech Republic.

Adenylosuccinate lyase deficiency is a rare autosomal recessive disease of purine metabolism affecting predominantly central nervous system. Although spectrum and severity of clinical symptoms overlaps, three forms of ADSL deficiency - severe neonatal, severe childhood and mild myopathic - can be distinguished clinically based on onset and severity of symptoms and biochemically on diverse ratios of accumulating succinylpurines in body fluids (SAdo/SAICAr ratio). The pathogenic mechanisms leading to the development of symptoms and underlying the phenotypic and biochemical heterogeneity remain unknown. We introduced a complex diagnostic system for ADSL deficiency based on metabolite profiling, enzyme activity measurements, mutation analysis and recombinant protein characterisation. So far we have analysed 22 patients from 18 families (8 Czech, 7 Poland, 5 Germany and 2 US), identified 16 ADSL mutations and cloned, expressed, purified and characterized catalytic properties of corresponding recombinant wild type and mutant ADSL proteins. We found evidence that residual enzyme activity, calculated as a mean of homoallelic activities, correlates with severity of phenotype. However, all the active mutant enzymes displayed proportional decrease in activity towards both substrates and no ground for the varied SAdo/SAICAr ratio was found. Based on these molecular data, prenatal diagnosis of 5 embryos was performed on genomic DNA isolated from chorionic villi. The analyses showed that 2 fetuses were heterozygotes for one mutation inherited from one parent and 3 fetuses inherited both mutations from their parents. We also investigated expression patterns of ADSL protein in available tissues from three deceased patients with R426H/Y114H, E376D/Y114H and R396H/Y114H genotypes. Compared to age matched controls, Western blot analysis showed in patients reduced amounts of ADSL in brain, heart, kidney and liver, but elevated amounts of ADSL in muscle. Based on these observations we hypothesize, that selective affection of central nervous system can be attributed rather to the toxic effects of accumulating succinylpurines then tissue specific involvement of ADSL function.

Analysis of Holoprosencephaly syndrome using array CGH indicates 32% of genomic rearrangements. *V. David[1,2], L. Rochard[1], C. Dubourg[1,2], I. Gicquel[1], MR. Durou[2], S. Jaillard[3], J. Mosser[1], V. Dupe[1], L. Pasquier[1,4], S. Odent[1,4], C. Bendavid[1]* 1) Fac de Medicine, UMR 6061 CNRS/Univ Rennes, Rennes, France; 2) Molecular Genetics, CHU Pontchaillou, Rennes, France; 3) Cytogenetics, CHU Pontchaillou, Rennes, France; 4) Medical Genetics, Hopital Sud, Rennes, France.

Holoprosencephaly (HPE) is the most common developmental brain anomaly in humans, usually associated with facial features. Our group focuses on patients with HPE and normal karyotype. Genetics of holoprosencephaly is complex: in our experience, mutations (18%) or deletions (8%) in the four main genes (SHH, ZIC2, SIX3 and TGIF) can explain about 26% of HPE cases. MLPA subtelomeric screening revealed 4% of additional complex rearrangements. In order to identify new candidate loci and thus novel candidate genes, we decided to screen HPE patients using Agilent CGH-array technology. 117 samples (68 fetuses and 49 live-borns children), with no karyotype alterations were tested using a unique male or female DNA as control. Out of these 117 samples, 37 presented with new rearrangements involving known or new potential HPE loci located on different chromosomes with 2 redundant regions. In a total of 117 patients, 37 chromosomal imbalances were detected (32%). These included 23 deletions, 9 duplications and 5 associated gains and losses. Detected alterations ranged from less than 1 gene to 17 Mb and were not further considered if they involved less than 3 consecutive spots on the array. None of these regions matched against copy number variations described in databases (TCAG database). Frequencies of alterations were higher in foetuses (70%) than in live-borns (30%). Thus, it appears that loss of genetic material represent severe alterations leading to more severe phenotypes. We are focusing on redundant rearrangements in order to identify new HPE genes; the prioritization of these genes is made using bioinformatics, expression in animal models during development and sequencing in patients.

A study of the frequency and haplotypes of SMA carrier alleles in the Ashkenazi Jewish population. *L. Liu, A. A. Mitchell, N. Cohen, R. J. Desnick, R. Kornreich, L. Edelmann* Dept Genetics/Genomic Sciences, Mount Sinai Sch Medicine, New York, NY.

Spinal muscular atrophy (SMA) is a common autosomal recessive neuromuscular disorder and has been reported to occur at a frequency of about 1 in 6000 liveborns. Mutations in the SMN1 gene on 5q13 are identified in the great majority of SMA patients. SMN1 resides in a region of the genome which has complex repetitive architecture and a highly homologous copy, SMN2, is located 1.4 Mb proximally on 5q13. Most mutations involve SMN1 copy number loss by either deletion or gene conversion events with SMN2 and consequently, carrier testing is performed using dosage sensitive methods. Approximately 1 in 40 individuals is a carrier for SMA; however, no data exists specifically for the Ashkenazi Jewish (AJ) population for which the frequencies of a number of disorders are high due to founder mutations. To determine the frequency of SMA carriers, we screened 692 individuals of AJ ancestry using MLPA. Fifteen SMN1 mutation carriers (1 in 46) were identified, and three major mutation groups were encountered; (1)SMN1 deletion, (2)SMN1 and SMN2 deletion (3)SMN1 exon 7/8 deletion. To determine whether these mutation groups represented founder alleles, we genotyped all 15 carriers and 25 AJ controls with 11 microsatellite markers that span 4.7 Mb on 5q13. In all three groups, a conserved haplotype of at least four consecutive alleles was identified. Haplotype A was found at estimated frequencies of 13% in Group 1, 8% in Group 2 and 2% in controls. Haplotype B, the most commonly identified haplotype in all groups, was found at estimated frequencies of 25% in Group 1, 36% in Group 2 and 12% in controls. Haplotypes A and B were not seen in Group 3. Haplotype C was found at estimated frequencies of 30% in Group 3, 14% in Group 2 and 5% in controls, but absent from Group 1. Interestingly, 4 of the 15 carriers did not fit into one of the three haplotype groups, which is likely to reflect the high denovo mutation rate at this locus. Our data support the existence of SMA founder alleles and represent the first rearrangement disorder for which AJ founder mutations have been identified.

Prioritization of Candidate Genes for Retinitis Pigmentosa. *K. F. Cribben[1], T. A. Braun[1], J. H. Fingert[1], V. C. Sheffield[1,2], E. M. Stone[1,2], T. L. Casavant[1], T. E. Scheetz[1]* 1) University of Iowa, Iowa City, IA; 2) Howard Hughes Medical Institute, Chevy Chase, MD.

We have previously developed a method for prioritizing gene candidates (NPCE) and utilized that method to successfully identify two novel genes for Bardet-Biedl syndrome. This method calculates a significance score for a set of genes based upon the network of pairwise correlations among the genes. This NPCE strategy provides a single representative value for a given network of gene correlations. The relative benefit for adding a gene to an existing network is then evaluated by assessing the significance score of that new network versus those networks derived from adding any other single gene. Each gene may then be prioritized based upon its relative contribution to the significance score. Our ability to prioritize the set of retinitis pigmentosa (RP) genes, however, varies greatly. This is due to the variable structure in the network of expression correlations among the RP genes, likely representing the increased complexity of RP. To address this lack of consistent structure, we iteratively reduced the RP network to the size of the BBS network (12 genes). The NPCE method was used to prioritize these 12 genes using a leave-one-out strategy. This resulted in a median prioritization in 11^{th} percentile of all genes, ranging from the 1^{st} to the 27^{th} percentile. This technique was applied to the set of linked RP intervals in human for which the disease gene is unknown, and identified several interesting candidates. We expect that the performance of the NPCE method can be increased significantly, particularly for the case of complex diseases. Several other expression data sets have yielded very promising preliminary results. The NPCE method is being extended to incorporate: (i) multiple expression data sets simultaneously, (ii) graph-theoretic approaches, and (iii) comparative genomic methods to utilized data sets from multiple species. The diffuse network structure of RP will be an excellent test-bed in developing the next generation of candidate gene prioritization methods needed to address the needs of truly complex diseases (diabetes, macular degeneration, etc).

Inherited abnormalities of unknown significance detected by aCGH and their implication in prenatal decisions, experience from an Israeli hospital. *H. Yonath[1,2], M. Berkenstadt[1], M. Frydman[1,2], B. Ghidoni- Ben-Zeev[1,2], E. Praas[1,2]* 1) Sheba Medical Ctr, Ramat Gan, Israel; 2) Sackler, TAU, Israel.

The increasing use of array CGH (aCGH) has refined our knowledge of chromosomal aberrations. In some of the cases the significance of the findings is unknown, especially when familial and rare. Many Israeli couples elect to perform invasive prenatal testing in order to assure a normal offspring. The indications include: advanced maternal age, abnormal US findings, previous abnormal child and parental concern. Increasing numbers of these samples are sent for aCGH analysis. When known deletion or duplication syndromes are detected by aCGH, the couples often elect to terminate the pregnancy. It is problematic when the significance is unknown. We present here four such families: Family #1: A young boy suffers from seizures, speech delay and compromised fine-motor skills. His brain MRI showed partial agenesis of the corpus callosum (CC). aCGH revealed a gain on 9p24.3. His healthy father has the same gain, and a normal brain MRI. The paternal grandfather, a normal male, had a single seizure at 63 years. Brain MRI revealed complete agenesis of the CC, and the same gain on chromosome 9. Family #2: An aCGH on a normal female fetus revealed a maternally inherited copy gain in Xp11.22. The first pregnancy was terminated due to a male fetus that had severe hydrops, DNA was extracted and saved. The girl is now 6 months old and developing well. Family #3: An aCGH preformed on a female fetus due to parental concern, revealed a maternally inherited 18p11.32 duplication. The mother is normal and healthy. Family #4: A female fetus' aCGH revealed deletions on 7p21.3 and 13q12.11. It was sent due to a previous son with cerebello-cerebral progressive atrophy, which has a deletion at 7p21.3. The father, who is healthy, has both deletions. Even after an in depth literature and database search, the clinical significance of these abnormalities is difficult to determine. These cases illustrate the problems encountered when interpreting certain familial aCGH abnormalities, especially when these cases are performed prenataly and the results are needed within a short time.

Dopamine Polymorphisms and Depressive Symptoms Predict Intake of Salty and Sweet Foods in a US Nationally Representative Sample of Adolescents and Young Adults. *TD. Agurs-Collins[1], BF. Fuemmeler[2]* 1) Health Promotion Research Branch, National Cancer Institute, Bethesda, MD; 2) Department of Community and Family Medicine, Duke University Medical Center.

Background: The brain mesolimbic dopaminergic system may play an important role in sensory pleasure of food, drugs and other rewards. Evidence suggests gene expression alterations in this system may mediate some of the rewarding effects of sucrose. Depression and emotional eating are associated with increased intake of sweet high energy-dense foods and salty high energy-dense foods. We examined the association between genotypes linked to dopaminergic pathways and depressive symptoms on predicting consumption of salty and sweet snack foods. **Methods:** A subsample of participants in Wave III of the National Longitudinal Study of Adolescent Health (n=1551) who provided DNA, an in-home 24-recall, and a measure of depressive symptoms were included in the analysis. Salty/high fat snack and sweet food indices were developed from the 24-hour recall. Depressive symptoms were measured using the Center of Epidemiologic Studies-Depression Scale (CES-D). Polymorphisms analyzed were dopamine transporter (DAT) and dopamine D2 receptor (DRD2). Multivariate linear regression analyses for genotype and depressive symptoms predicted consumption of sweet and salty snack foods. Models were stratified by gender and adjusted for age, parental education, race and body mass index (zBMI). Interactions between genotype*depressive symptoms were explored. **Results:** Significant main effects were observed for depressive symptoms among males consuming salty/high fat snack foods (p=0.01)and among females with the 10/10 allele relative to any 9 allele on salty/high fat snack foods (p=0.03). Significant DAT gene*depressive symptoms interaction was observed for females predicting consumption of sweet snack foods (p<.001)and a significant DRD2 gene*depressive symptoms interaction for males consuming salty/high fat snack foods (p=0.03). **Conclusions:** These findings suggest that genes associated with the dopaminergic system and depressive symptoms may play an important role in consumption of snack foods with possible gender differences.

Bayesian Algorithm for scoring polymorphic deletions from SNP data and genomewide scan for common CNVs.

S. Zollner[1,2], W. C. L. Stewart[1], K. Stephenson[1], P. Zhang[1], M. Mcinnis[2] 1) Dept Biostatistics, Univ Michigan, Ann Arbor, MI; 2) Dept Psychiatry, Univ Michigan, Ann Arbor, MI.

Copy Number variations (CNVs) in the human genome provide exciting candidates for functional polymorphisms affecting complex trait phenotypes. Efforts are underway to generate a CNV-map by identifying and cataloging all common CNVs in the human genome. Thus, association of CNV carrier status and diseases status in case-control studies can be assessed. One approach to score such common CNVs is to evaluate the signal intensity of SNP genotyping assays. However, such analyses are hampered by a lack of appropriate statistical methods. Here we present a novel statistical method that is designed to perform such inference, assess this method using a known CNV and apply it to a large sample of genomewide SNP genotyping data. Using a bayesian algorithm we calculate the posterior probability for the carrier status, jointly analyzing genotype information and hybridization intensity. We model the signal intensity as a mixture of normal distributions, allowing for locus-specific and allele-specific distributions estimated using an EM algorithm. Our model accounts for genotyping error and uncertainty about the boundaries of an assessed CNV. We test the method using a previously described common deletion on 8q24 in a sample of 3512 individuals. We unambiguously inferred 172 heterozygous and 1 homozygous deletion carrier. We assessed the power of this algorithm to detect shorter CNVs by sub-sampling from the SNPs covered by this deletion, We demonstrate that our EM algorithm produces precise estimates for CNVs covering 7 or more SNPs, thus providing a better resolution than commonly used hidden Markov models. We apply the method to a population sample of 1600 individuals sequenced with the Affymetrix 6.0 chip to assess carrier status for 15466 CNVs collected in the database of genomic variants. In our analysis we use both the genotype probes and the CNV probes on the Affy 6.0 chip. We thus describe the utility of large collections of CNVs for association mapping.

***CFTR* screening in infertile couples candidate for Assisted Reproductive Techniques (ART).** *A. Saluto, A. Murro, A. Celeste, A. Marongiu, G. Voglino* Laboratory of Molecular Genetics - PROMEA S.p.A - Turin, Italy.

Cystic Fibrosis (CF) is a multi-system autosomal recessive genetic disorder caused by mutations in the Cystic Fibrosis Transmembrane Regulator *(CFTR)* gene primarily affecting Caucasian populations. With an incidence of about 1 in 2500 live births it is the most common life-shortening disease. Thus far, more than 1600 mutations in the *CFTR* gene have been identified; the great majority are rare and present in only one or a few CF patients. The most common mutation is F508, whose prevalence varies among populations of different geographical and ethnic backgrounds, accounting approximately for the 70% of the mutations in European patients. Our aim was to evaluate the frequency of *CFTR* mutations in infertile couples referred to our center for Assisted Reproductive Techniques (ART) (either IVF-ICSI or IUI). *CFTR* gene screening was performed according to Italian guidelines for genetic tests in infertile couples (C. Foresta et al, 2002). We analysed 2888 infertile couples in the last five years; molecular analysis of the *CFTR* gene was performed on genomic DNA extracted from peripheral blood lymphocytes by using OLA assay (Cystic Fibrosis Genotyping Assay, Abbott Laboratories, Germany). The 3.6% (208/5776) of the tested patients showed the presence of a *CFTR* gene mutation; these patients underwent a genetic counseling to inform the couple of the genetic risk for having a baby with CF. The prevalence of 1/28 cystic fibrosis heterozygous carriers we detected is in agreement with previous studies for the Italian population. 131/208 mutations (63%) were F508 that was confirmed using a rapid test developed in our laboratory based on fluorescent fragment analysis of *CFTR* exon 10; the same test is utilized for particular PGS (Prenatal Genetic Screening) cases using QF-PCR technique. To our knowledge this is one of the largest screening of *CFTR* gene in Italian infertile couples and we do not show higher prevalence of mutations in infertile cases compared with the general Caucasian population.

Genetics of VEGF serum level variation in human: importance of VEGF gene polymorphisms. *D. Ruggiero[1], R. Sorice[1], C. Bellenguez[2], T. Nutile[1], G. Fardella[1], M. Aversano[1], C. Bourgain[2], M. Ciullo[1]* 1) Institute of Genetics and Biophysics - CNR, Naples, Italy; 2) INSERM U535, Villejuif, France.

Vascular Endothelial Growth Factor (VEGF) is a potent mediator of angiogenesis and vascular permeability, having a key role in both physiological and pathological angiogenesis. In order to identify QTLs influencing VEGF serum levels, we performed a genome wide linkage analysis using a sample of individuals randomly chosen in Campora, an isolated population in South Italy. VEGF serum levels were measured using the ELISA method in a sample of 656 individuals. 627 individuals out of the 656 were all related through a 3049-member pedigree and genotyped for 1122 microsatellites on the genome (average marker spacing of 3.6cM). The heritability for VEGF serum levels was estimated to be 0.86 after adjusting for age. To perform linkage analysis we broke the large genealogy using the GREFFA method through a multiple splitting approach recently proposed by Bellenguez and coll. With the regression-based linkage statistics proposed by Sham and collaborators (2002), we detected a strong linkage on chromosome 6p12.3 (LOD=10.07) at marker D6S459. This linkage signal exactly corresponds to the location of the VEGF gene. Next, the coding and regulatory regions of the VEGF gene were sequenced in a sample of 42 individuals. 33 polymorphisms were identified, 24 of which having a MAF>0.05. The association between these 24 SNPs and VEGF levels was tested using the GTAM test, that also corrects for relatedness between individuals through the genealogical information. After correction for the number of independent tests performed, a significant association was detected between three of the 24 SNPs ($p<0.001$) and VEGF serum levels. To assess if the linkage signal detected on chromosome 6 could be explained by these associations, linkage analysis was carried out taking into account the effect of the SNPs on the trait. A lower linkage peak (LOD=6.457) was obtained, showing that 35% of the linkage is explained by the identified variants. With this work therefore, we show that isolated populations, allowing both linkage and association analyses, could be a powerful approach in complex trait studies.

Validation Study of Candidate Gene SNP Genotyping Protocol. *A. Kureichyk, S. Wendell, ML. Marazita* School of Dental Medicine, University of Pittsburgh.

The single nucleotide polymorphism (SNP) genotyping of candidate genes using commercial fluorescent PCR assay systems is utilized to investigate genetic associations. We sought to validate a SNP genotyping protocol, adapting the recent advances in saliva DNA extraction, PCR based human DNA quantification, sample handling, and PCR based genotyping. Methods Human DNA from saliva was extracted and handled under separate PCR hoods. Barcode labels and electronic file conversions were used for sample handling and software analysis. The RNaseP Taqman assay from Applied Biosystems, (ABI) was used to quantify the human component of the saliva DNA. Genotyping with five SNP assays was conducted on three concentrations (0.45, 4.5, and 22.5ng) of 15 individual DNA samples in 5ul reactions. This was done in triplicate on separate days totaling 9 genotype calls per sample between three concentrations and 675 accumulative genotype calls. PCR quantification and genotyping were analyzed on the ABI 7900 using SDS software in absolute quantification or allelic discrimination mode respectively. Genotype calls were assessed using SDS software in a variety of settings including manually adjusted autocalls. Restriction enzyme digests were conducted on two SNP assays to confirm fluorescent PCR based genotype calls. Results Tight genotype clustering was seen using DNA normalized by RNaseP assay. The level of undetermined genotypes (45/675, 6.7%) was minimal with successful genotyping in at least one replicate at the same DNA concentration. Two incorrect genotypes (2/675, 0.3%) were seen in the same SNP and represented the only homozygous minor allele genotypes for this SNP, ruling out cross contamination. The 4.5ng DNA concentration was optimal with 4 out of 225 (<2%) undetermined genotypes. Independent PCR and restriction enzyme digest in two SNP regions confirmed accurate genotyping. Conclusions The assessed protocol for candidate gene genotyping indicated a low level of undetermined genotypes (6.7%) and incorrect genotypes (<0.3%). The 0.45ng/5ul DNA concentration was optimal for genotype clustering and successful calls (>98%). All genotypes for two SNPs were confirmed by independent restriction enzyme digest. NIH Grant # DE014899.

Association of Sporadic ALS with Candidate Genes and Environmental Risk Factors: The Genes and Environmental Exposures in Veterans with ALS (GENEVA) Study. *S. Schmidt[1], K. Allen[1,2], J. Rimmler[1], V. Loiacono[1], C. Stanwyck[1], C. Williams[1], H. Munger[1], M. Hauser[1], E. Oddone[1,2]* 1) Dept Medicine, Duke Univ Med Center, Durham, NC; 2) VA Medical Center, Durham, NC.

An increased risk of amyotrophic lateral sclerosis (ALS) has been reported for US military veterans. Head injury and cigarette smoking have been implicated as risk factors for developing ALS, and both are more common in military personnel, compared to civilians. Recent genome-wide association studies have proposed DPP6 and ITPR2 as potential ALS susceptibility genes, but the respective SNPs (rs10260404, rs2306677) have not yet been examined in a veteran study population. In conjunction with the National Registry of Veterans with ALS, we have enrolled 617 patients with motor neuron disease (85% ALS, 98% male, 93% white) and 445 controls (94% male, 91% white) into the GENEVA case-control study to date. DNA samples from 852 cases and 202 controls have been genotyped thus far; results will be updated by adding 280 case and 190 control samples. We analyzed the association between head injury, cigarette smoking, SNP genotypes and ALS risk in the entire study population and the subset of incident cases. While the number of head injuries requiring medical attention was not significantly associated with ALS risk, an older age at the time of the last head injury conferred an increased risk. Relative to veterans without any head injuries, those with an age at last injury below 30 years, 30-40 years and >40 years, respectively, had an odds ratio (OR) for ALS of 0.99 (95% CI 0.64-1.56), 1.78 (0.84-3.80) and 2.44 (1.20-4.93) (p(trend)=0.01, 204 incident cases, 410 controls). There was no significant association between cigarette smoking and ALS risk. The minor allele frequencies of rs10260404 and rs2306677 in veteran controls were similar to those reported for civilian populations, but these SNPs were not associated with ALS risk in our sample. We conclude that head injuries may make a moderate contribution to the increased risk of ALS in US veterans, and will examine if coding variants in the apolipoprotein E (APOE) gene modify this relationship.

Left ventricular noncompaction is frequently associated with sarcomere protein gene mutations. *S. Klaassen[1], S. Probst[1], E. Oechslin[2], B. Gerull[1], F. Berger[3,4], R. Jenni[5], L. Thierfelder[1]* 1) Max-Delbrueck-Center for Molecular Medicine, Berlin, Germany; 2) Toronto General Hospital, Toronto, Canada; 3) German Heart Institute Berlin, Berlin, Germany; 4) Pediatric Cardiology, Charité, Berlin, Germany; 5) University Hospital Zürich, Zürich, Switzerland.

Introduction- Left ventricular noncompaction (LVNC) has recently been recognized as a primary cardiomyopathy. It is characterized by a severely thickened, two-layered myocardium, numerous prominent trabeculations, and deep intertrabecular recesses. Recently, mutations in genes encoding sarcomere proteins -myosin heavy chain (MYH7), -cardiac actin (ACTC), and cardiac troponin T (TNNT2) were shown to be associated with LVNC. We report on the frequency of mutations in sarcomere protein genes in a cohort of patients with isolated LVNC. Methods and Results- Mutational analysis of 8 sarcomere protein genes was carried out in a cohort of 63 unrelated Caucasian adult probands (mean age, 40 years) with LVNC and absence of other congenital heart anomalies. Denaturing high performance liquid chromatography (DHPLC) analysis and direct DNA sequencing were performed. Heterozygous mutations were identified in 18 of 63 samples (29%). Two new disease-genes were identified: cardiac myosin-binding protein C (MYBPC3) and -tropomyosin (TPM1). 5 mutations were found in MYBPC3, and 2 in TPM1. The LVNC phenotype was characterized by a variable adult-onset of symptoms such as heart failure and systemic emboli. Younger affected individuals were diagnosed because of family screening and were usually clinically asymptomatic. MYH7 was the most prevalent disease gene and accounts for 13% of cases followed by MYBPC3 as the second most frequent disease gene (8%). Conclusions- Mutations in sarcomere protein genes account for a significant proportion of cases of isolated LVNC in this cohort (29%). We describe the first mutations in MYBPC3 and TPM1 associated with isolated LVNC. These findings confirm that LVNC belongs to the spectrum of cardiomyopathies caused by molecular defects of the sarcomere and open new perspectives for genetic investigations and counseling.

An Overview of the Literature on Genetic Epidemiology of Infectious Disease. *R. Waltenburg[1,2], W. Yu[1], M. Gwinn[1], M. Khoury[1]* 1) National Office of Public Health Genomics, Centers for Disease Control and Prevention, Atlanta, GA; 2) Epidemiology Department, Rollins School of Public Health, Emory University, Atlanta, GA.

Background: Increasingly, host genomics has been explored to explain heterogeneity in susceptibility, severity and treatment response to infection. The Human Genome Epidemiology (HuGE) Published Literature database is a searchable online knowledge base comprised of population-based epidemiology studies of human genes. The database has been curated by the National Office of Public Health Genomics at the Centers for Disease Control and Prevention since October, 2000. The objective of this study was to characterize the HuGE infectious disease literature. **Methods:** On May 21, 2007 the HuGE Literature Finder was queried to identify studies of infectious disease with a publication date between 2001 and 2006. A 20% simple random sample by year was obtained (n=328) and 284 articles met inclusion criteria. **Results:** The number of publications increased each year from 2001 through 2006. Most studies were observational, with 93% exploring gene-disease associations and 23% examining gene-environment interactions. 69% examined disease susceptibility, while 29% examined disease severity. Forty-five percent of all studies incorporated some type of chronic outcome (i.e., chronic infection, chronic sequelae, carcinoma), with 14% of all articles studying cancer as an outcome. The mean number of genes per article was 2.1 (range 1-45). *TNF*, *IL10*, *HLA-DRB1*, *IL1B*, *CCR5*, *HLA-DQB1*, *IL6*, *IL1RN* and *IFNG* were the most commonly referenced genes. Twenty percent of all studies performed haplotype analyses of which half examined non-HLA genes. The most frequently studied pathogens were human immunodeficiency virus, hepatitis C virus, *Helicobacter pylori*, *Mycobacterium tuberculosis* and hepatitis B virus. Only 5% of studies incorporated pathogen characteristics into the analysis. **Conclusion:** Research into the contribution of human genomics in the study of host-pathogen interactions is becoming increasingly common, but much work remains to be done.

Genomic deletions/duplications in patients with esophageal atresia/tracheoesophageal fistula and VACTERL association. *B. J. Kim[1], H. Zaveri[1], E. M. de Jong[6], J. F. Felix[6], C. J. Fernandes[2], A. Johnson[3], K. P. Lally[4], D. Tibboel[6], A. de Klein[5], B. Lee[1,7], D. A. Scott[1]* 1) Mol & Hum Gen; 2) Pediatrics; 3) OB & Gyn, Baylor College of Medicine, Houston, TX; 4) Ped Surg, U of Texas Med School, Houston Texas; 5) Clin Genet; 6) Paed Surg Erasmus M.C. Retterdam, Netherlands; 7) Howard Hughes Med Ins.

Esophageal atresia/tracheoesophageal fistula (EA/TEF) is a life-thretening birth defect that affects 1:3,500 live births. Although EA/TEF can occur in isolation, approximately 50% of cases occur with additional anomalies. VACTERL (Vertebral, Anal, Cardiac, Tracheo-Esophageal Fistula, Renal, Limb) association is found in approximately 10% cases and is defined by the presence of at least three anomalies. The sporadic nature of EA/TEF makes a linkage-based approach to gene identification impractical. In an alternative approach, we are using a positional candidate approach based on chromosomal data to localize and identify the genes that cause or predispose to the development of EA/TEF. A review of published cases revealed 10 chromosomal regions that are recurrently deleted/duplicated in EA/TEF. We hypothesize that each of these regions harbors one or more EA/TEF-related genes. To identify new chromosomal regionsand refine those previously reportedwe screened a cohort of 75 patients with EA/TEF for cryptic deletions/duplication in affected individuals using high density genome-wide array comparative genome hybridization. Although the majority of deletions/duplication had been previously identified in normal controls, over 100 rare genomic variants were identified. These rare variants affect a variety of genes that play important roles in cell migration, adhesion, differentiation, proliferation, and signaling. We are presently using quantitative PCR to determine the inheritance pattern of these deletions/duplication within families. Although de novo changes are considered more likely to be causative, the 2% recurrence risk seen in EA/TEF and VACTERL suggests that the majority of cases may result from a combination of inherited changes affecting important developmental pathways combined with environmental stressors such as maternal diabetes.

Pharmacogenetics of low dose naltrexone treatment in primary progressive multiple sclerosis. *F. Martinelli Boneschi[1], M. Gironi[1,2], P. Sacerdote[3], F. Esposito[1], S. Lupoli[1], C. Solaro[4], M. Zaffaroni[5], R. Cavarretta[2], L. Moiola[1], S. Bucello[1], M. Radaelli[1], S. Franchi[3], V. Martinelli[1], R. Nemni[2], G. Martino[1], G. Comi[1]* 1) Institute of Experimental Neurology (INSPE), and Department of Neurology, San Raffaele Scientific Institute, Via Olgettina 58, 20132, Milan; 2) Fondazione Don Carlo Gnocchi, Milan; 3) Department of Pharmacology, University of Milan, Milan, Italy; 4) Department of Neurology, ASL 3 genovese, Genoa, Italy; 5) Multiple Sclerosis Study Center, Hospital of Gallarate, Gallarate (VA), Italy.

Primary progressive multiple sclerosis (PPMS) represents a rare variant of disease, characterized by a progression of neurological symptoms from the beginning, for which no treatment is available. We performed a sixth-month phase-II multicentre pilot trial with low dose Naltrexone (LDN) in 40 patients with PPMS. The primary end points of the study were safety and tolerability, while secondary outcomes were its efficacy on spasticity, pain, fatigue, depression, and quality of life. Since the rationale of the treatment is related to an increase in beta-endorphins, intracellular protein levels of beta-endorphins (BE) in PBMC using a radioimmunoassay procedure and mRNA levels and allelic variants of the Mu-opiod-receptor gene (OPRM1) were analysed in all patients at differen follow-up. At the end of the trial, we measured a significant reduction of spasticity which was paralleled by a significant increase in beta-endorphin levels. However, no association was found between *OPRM1* variant (Asn40Asp SNP) and mRNA levels of beta-endorphins and response to treatment, defined as an amelioration in spasticity. Thirty-one patients (79.5%) were homozygous, while 8 (20.5%) heterozygous for the more common A variant of *OPRM1* gene, and no association was found between allelic variants and symptomatic responsiveness (p:ns; OR and 95% C.I. of G carriers and efficacy on spasticity: 3.11; 0.6-16.8). A larger study including more patients is ongoing.

DNA Methylation Profiles in Monozygotic and Dizygotic Twins. *Z. Kaminsky[1,6], T. Tang[1,6], S.-C. Wang[1,8], C. Ptak[1,6], G. Oh[1,6], S. Ziegler[1,6], A. Wong[1,6], L. A. Feldcamp[1,6], C. Virtanen[2], J. Halfvarson[3,7], C. Tysk[3,7], A. F. McRae[4], P. M. Visscher[4], G. W. Montgomery[4], I. I. Gottesman[5], N. G. Martin[4], A. Petronis[1,6]* 1) Ctr Addiction & Mental Health, Toronto, ON, Canada; 2) University Health Network Microarray Centre, Toronto, Ontario, Canada 4. 5; 3) Division of Gastroenterology, Department of Medicine, Örebro University Hospital, Örebro, Sweden; 4) Queensland Institute of Medical Research, Brisbane, Australia; 5) University of Minnesota, Minneapolis, Minnesota, USA; 6) University of Toronto, Toronto, Ontario, Canada; 7) School of Health and Medical Sciences, Örebro University, Örebro, Sweden; 8) Institute of Systems Biology and Bioinformatics, National Central University, Chungli, Taiwan.

Comparison of phenotypic similarities and differences in monozygotic (MZ) and dizygotic (DZ) twins has provided the basis for molecular genetic and epidemiological studies in human diseases and normal traits. In the wake of increasing evidence that epigenetic factors can contribute to phenotypic outcomes, we performed a DNA methylome analysis of MZ twins in white blood cells (WBC), buccal epithelial cells, and gut (rectum) biopsies (N=57 pairs in total) using 12K CpG island microarrays. The detected DNA methylation differences provided the basis for the first annotation of epigenetic metastability of ~6,000 unique genomic regions in MZ twins. We also compared the degree of DNA methylation difference in WBC and buccal epithelial cells from 39 pairs of MZ twins to 40 pairs of DZ twins. DZ co-twins exhibited significantly higher epigenetic difference compared to the MZ co-twins in buccal cells ($p=1.2x10^{-294}$). While such higher epigenetic discordance in DZ twins can result from DNA sequence differences, our in silico SNP analyses and comparison of methylomes in inbred vs. outbred mice favour the hypothesis that this is due to epigenomic differences in the zygotes. This study suggests that molecular mechanisms of heritability may not be limited to DNA sequence differences.

Natural Selection Targets Innate Immunity Genes: the Paradigm of the Human Toll-Like Receptor Family. *L. B. Barreiro[1,2], H. Quach[1], E. Patin[1], G. Laval[1], C. Bouchier[3], M. Tichit[3], O. Neyrolles[4], B. Gicquel[4], J. R. Kidd[5], K. K. Kidd[5], A. Alcais[6,7], L. Abel[6,7], J. L. Casanova[6,7], L. Quintana-Murci[1]* 1) Institut Pasteur, Human Evolutionary Genetics, CNRS, URA3012, Paris, France; 2) University of Chicago, Department of Human Genetics, Chicago, USA; 3) Plate-forme Génomique, Pasteur Genopole, Institut Pasteur, Paris, France; 4) Unité de Génétique Mycobactérienne, Institut Pasteur, Paris, France; 5) Department of Genetics, Yale University School of Medicine, New Haven, USA; 6) Laboratory of Human Genetics of Infectious Diseases, Necker Medical School, Paris, France; 7) University Paris René Descartes, Necker Medical School, Paris, France.

Toll-like receptors (TLRs) are thought to be essential for host defense by sensing and initiating innate and adaptive immune responses against microbes. Here, we characterized the levels of genetic variation in the ten human TLRs in a natural ecosystem governed by natural selection, namely in a panel of healthy individuals representative of the general population worldwide. We found that the nucleic acid sensors TLR3, TLR7, TLR8 and TLR9, which are principally involved in viral recognition, have evolved under strong purifying selection, with mutations altering the encoded proteins proscribed. Conversely, selective constraints on the remaining six TLRs, which detect non nucleic acid microbial products on the cell surface, have been much more relaxed, with higher rates of missense and nonsense mutations tolerated. However, although amino acid-altering variants of these genes may be tolerated, weak negative selection precludes increases in the frequency of these variants in the general population. Finally, we identified two haplotypic backgrounds in the region encompassing TLR10-TLR1-TLR6 showing clear signs of positive selection in Europeans and East-Asians, indicating the presence of variants conferring a selective advantage to host survival. Our evolutionary data indicate that the different members of TLR family differ in their ecological relevance and increase our understanding of how variation in these genes results in different contributions to the outcome of infectious diseases.

A new wide-genome and high resolution approach for detecting methylation profiles through simulations and modeling. *L. Pantano[1,2,3], R. Rabionet[2], X. Estivill[2,3], C. Notredame[1]* 1) Comparative bioinformatics, CRG, Barcelona, Spain; 2) Genes and Diseases, CRG, Barcelona, Spain; 3) Universidad Pompeu Fabra, UPF, Barcelona, Spain.

Epigenetics refers to heritable phenotypic alterations in the absence of DNA sequence changes, and DNA methylation is one of the extensively studied epigenetic alterations. Genomic DNA methylation profiles exhibit substantial variation within the human population, with important functional implications for gene regulation through its ability to induce locally condensed chromatin structure. Promoter hypermethylation is known to cause stable silencing of associated genes and plays an important role for both normal and disease development. We model and simulate in order to benchmark a novel wide-genome and high-resolution approach for detecting the genome methylation profile assuming an open/close model for CpG Island. Based on our finding, we propose a cost-optimized strategy for mammalian methylome projects.

Inferring human admixture history using a copying model. *G. Hellenthal[1], D. Falush[2], S. Myers[1]* 1) Dept Statistics, Univ Oxford, Oxford, United Kingdom; 2) Microbiology, University College Cork, Cork, Ireland.

We have developed a novel statistical method to characterize the admixture events by which individual populations were formed. The method is applied to recently published genome-wide SNP data for the Human Genome Diversity Panel (Science 319, 1100-1104). We show that it is possible to date admixture events that took place between 5 and 500 generations ago. We are able to reconstruct a number of events supported by strong historical evidence, including the wave of Bantu admixture, ancient African admixture into middle eastern populations, and ancient secondary East Asian migrations into Melanesia. We also analyze signals relating to more ancient and historically less well understood events, several of which are relevant to major anthropological controversies.

Genome-Wide Linkage Analysis of Platelet Phenotypes in White and African American Families with Coronary Artery Disease. *R. A. Mathias[1], Y. Kim[1], L. Yanek[2], J. E. Herrera-Galeano[2], L. C. Becker[2], D. M. Becker[2], A. F. Wilson[1]* 1) IDRB/NHGRI/NIH, Genometrics Section, Baltimore, MD; 2) Johns Hopkins Medical Institutions, Baltimore, MD.

Background: The inability of aspirin (ASA) to adequately suppress platelet aggregation is associated with future risk of myocardial infarction, stroke, and cardiovascular death; and genetic variation may be responsible for ASA responsiveness. In this study, we performed a genome-wide linkage scan for platelet phenotypes before and after ASA treatment (i.e. pre-ASA, post-ASA or change-after-ASA). Methods: Clinical data on 37 agonist-induced platelet function phenotypes were evaluated in 1231 white and 846 black healthy subjects with a family history of premature CAD before and after a 2-week trial of ASA (81 mg/day). There were 243 black and 398 white pedigrees, respectively. Principal components analyses were run separately for whites and blacks on the phenotypes adjusted for age, sex, diabetes, hypertension, smoking, LDL cholesterol, fibrinogen, and body mass index. Nine factors were identified for the pre-ASA variables, and 8 factors each were identified for the post-ASA and change-after-ASA variables. Genotyping was performed at deCODE Genetics with the standard deCODE 550 STR marker set (average spacing= 8cM). Linkage analysis was performed with the Hasemen-Elston regression approach in SAGE (v 5.1.0) for each factor and each STR within race. Results: Three loci in blacks and one in whites were significant at the 0.0001 level. A 5cM region in blacks on 1q41-42 had a pleiotropic effect (i.e. linkage to 5 platelet-rich plasma [PRP] or whole blood aggregation factors to different doses of collagen, all either post-ASA or change-after-ASA); and a 5cM region in whites on 4p12 had significant linkage to PRP aggregation to high doses of collagen post-ASA. Conclusion: Several genomic regions show significant evidence for linkage to agonist-induced platelet function phenotypes in the context of aspirin response. The strongest evidence appears to be for post-ASA or change-after-ASA aggregation to collagen as an agonist in both blacks and whites, although in different regions for the two races.

Transglutaminase-1 mutations and genotype-phenotype investigations of 104 patients with ARCI in the USA. *S. Farasat[1], M. H. Wei[1, 2], O. Toure[1], M. L. Herman[1], D. J. Liewehr[3], S. M. Steinberg[3], S. J. Bale[4], P. Fleckman[5], J. R. Toro[1]* 1) Genetic Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, Rockville, MD 20892, USA; 2) Basic Research Program, SAIC-Frederick Inc., Frederick, MD 21702, USA; 3) Biostatistics and Data Management Section, Center for Cancer Research, National Cancer Institute, NIH, Bethesda, MD 20852, USA; 4) GeneDx, Inc, Gaithersburg, MD 20877, USA; 5) Division of Dermatology, University of Washington, Seattle, WA 98195, USA.

Autosomal recessive congenital ichthyosis (ARCI) is a rare hereditary disorder of keratinization. Mutations in the transglutaminase-1 (TGM1) gene, which encodes for the transglutaminase-1 (TGase-1) enzyme, are one of the causes of ARCI. We characterized the TGM1 mutation spectrum, and investigated genotype-phenotype correlations in 104 patients with ARCI ascertained through the USA National Registry for Ichthyosis. Germline mutations in TGM1 were identified in 55% (57/104) of ARCI patients. Arginine residues were mutated in 39% (22/57) of all patients and 54% (20/37) of patients with missense mutations. Fifty-five percent (12/22) of missense mutations were within CpG dinucleotides. Our genotype-phenotype investigation found that ARCI patients with TGM1 mutations were significantly associated with presence of collodion membrane at birth (p=0.006), ectropion (p=0.001), plate-like scales (p=0.005), and alopecia (p=0.001). Patients with at least one mutation predicted to truncate TGase-1 were associated with severity of hypohidrosis (p=0.001) and overheating (p=0.0007) at onset of symptoms compared with patients with only TGM1 missense mutations. Patients with missense mutations in the catalytic core were associated with eclabium compared with patients with mutations predicting to truncate TGase-1 (p=0.003). We developed a model predicting that patients with collodion membrane, alopecia, and/or eye problems were about four times more likely to have TGM1 mutations than patients without these findings. This study expands the TGM1 mutation spectrum and shows that TGM1 is the main causative gene for ARCI. There is a high frequency of mutated arginines in TGase-1 associated with ARCI.

Associations and interactions between a network of dopaminergic gene polymorphisms in schizophrenia: a follow-up in an Indian sample. *V. Kodavali[1], P. Semwal[4], M. E. Talkowski[1], J. Wood[1], S. Deshpande[3], B. K. Thelma[4], V. L. Nimgaonkar[1,2]* 1) Dept Psychiatry, Univ Pittsburgh, Pittsburgh, PA; 2) Dept of Human Genetics, Univ of Pittsburgh, Pittsburgh, PA; 3) Dept of Psychiatry, Dr. RML Hospital, New Delhi, India; 4) Dept of Genetics, Univ of Delhi, South Campus, New Delhi, India.

We have earlier evaluated the hypothesis that dopaminergic polymorphisms are risk factors for schizophrenia (SZ) in Caucasian samples from the USA and Bulgaria (Talkowski, et al, Hum. Mol. Genet. 2008). The most promising associations are detected with SLC6A3 (alias DAT), DRD3, COMT and SLC18A2 (alias VMAT2). Here we present the follow-up analyses in the collaborative Indian trio sample (n=601 families). Unscreened cord blood samples (n= 520) were collected from live birth at Lok Nayak Hospital, New Delhi. The SZ diagnoses were based on DSM IV criteria, similar to the US sample. Informed consent was obtained from the probands and family members at Ram Manohar Lohia Hospital, New Delhi. Snplex and SnapSHOT assay methods were employed for SNP genotyping. Our goals were, a) to seek replication at SNP level based on our earlier work in the US and Bulgarian sample, b) to understand the linkage disequilibrium (LD) patterns in the Indian sub-population and c) to test the epistatic interactions reported. We have evaluated a total of 41 SNPs. Suggestive associations were detected in the same direction reported earlier at SLC18A2 (rs363338, trends test p = 0.075). At DRD3 and SLC6A3, trends were noted with the opposite alleles (respectively, rs324030, p = 0.084; rs403636, p = 0.058).We also observed additional SNPs with suggestive associations at SLC18A2 (rs363399, rs10082463 and rs363285, p 0.1). The LD patterns were similar to the US and Bulgarian samples. Earlier reported epistatic SNP interactions at all four genes between seven locus pairs (p0.05), were not noted. However, we observed other epistatic interactions, the majority being between SNPs at SLC6A3 and COMT. They need to be explored further at a functional level. Overall, we suggest the importance of DA genes and their interaction, as risk factors for SZ in the Indian sample.

Co-regulation of ancestral pseudoautosomal genes in the mouse. *M. A. Levy[1,4], A. D. Fernandes[1,2], D. C. Tremblay[1,4], C. Seah[3,4], N. G. Bérubé[1,3,4]* 1) Department of Biochemistry, University of Western Ontario, London, Canada; 2) Department of Applied Mathematics, University of Western Ontario, London, Canada; 3) Department of Paediatrics, University of Western Ontario, London, Canada; 4) Children's Health Research Institute, Lawson Health Research Institute, London, Canada.

The X and Y chromosomes in mammals are morphologically distinct. However, eutherians retain homologous regions at the tips of the X and Y referred to as pseudoautosomal regions (PARs). The PARs play a critical role in the obligatory X-Y crossover during male meiosis. Genes that reside in the PAR are exceptional in that they are rich in repetitive sequences and undergo a very high rate of recombination. Murines lack the large PAR regions seen in other eutherians. Remarkably, murine PAR1 homologs have translocated to various autosomes, reflecting the complex recombination history during the evolution of the mammalian X chromosome. Here we show that the SWI/SNF chromatin remodeling protein ATRX controls the expression pattern of ancestral PAR genes that have translocated to autosomes in the mouse. We propose that the ancestral PAR genes share a common epigenetic environment, originating from their ancestral location in the PAR, which allows ATRX to control their expression.

Genome wide association study for Fibromuscular Dysplasia(FMD). *J. Yang[1], M. Nalls[2], B. Traynor[2], D. Hernandez[2], N. Washecka[2], B. Griswold[1], L. Sloper[1], A. Singleton[2], N. B. McDonnell[1]* 1) LCI, NIA/NIH, Baltimore, MD; 2) LNG,NIA/NIH,Bethesda,MD.

Fibromuscular dysplasia is a heterogeneous disorder characterized by angiopathy of the arteries, which predominantly affects women in mid-life, resulting in stenosis, dissection or aneurysm. It may involve renal arteries, carotid, vertebral, coronary and abdominal vessels. Autosomal dominant inheritance has been suggested, however no genes have been identified to date. On detailed clinical examination of a cohort of 69 patients seen at the National Institute on Aging, features of connective tissue dysplasia as exemplified by joint hypermobility, scoliosis, pectus deformity, pes planus and high myopia were frequently noted, suggesting a defect in the extracellular matrix pathways. In order to elucidate the genetic factors underlying this disorder, we performed genome wide association study in 46 sporadic cases and 540 controls utilizing the Illumina HumanCNV370-Duo BeadChip and HumanHap550 Beadchip. By permutation analysis, we have identified a number of marker SNPs with significant differences between cases and controls. The candidate genes include CDH12, EXOC2, PACRG, HECW1, ZAN, CSMD1, SRA and DIP2B with p scores <10-6. Although the disorders has been suggested to be dominant, we did homozygosity analyses for our samples, our results show chromosome 4, 5, 12 could include some specific overlapping homozygous runs in FMD patients.

Delineating Williams-Beuren Syndrome Chromosomal Region Using High-density Targeted Array CGH. *X. Hu[1,2], D. Mercer[2], T. Narumanchi[2,3], H. Andersson[2,3], M. Li[1,2,3]* 1) Louisiana Cancer Research Cons, Tulane Univ Sch Medicine, New Orleans, LA; 2) Hayward Genetics Center,Tulane Univ Sch Medicine, New Orleans, LA; 3) Dept. of Pediatrics; Tulane Univ. Sch. Med, New Orleans, LA.

Williams-Beuren syndrome (WBS) is caused by a heterozygous deletion of contiguous genes at 7q11.2. Three large region-specific low copy repeat elements (LCRs A, B, and C) located in the proximal region of chromosome 7q are thought to be responsible for the high frequency of deletions in this region. These LCRs also hinder the molecular characterization of deletions in different WBS patients and the phenotype/genotype correlation of the syndrome. We attempt to use a novel technology, high density targeted array CGH, to delineate the deletions in 6 patients with WBS. In this study, we constructed a custom designed targeted array that covers the commonly deleted chromosomal region and flanking regions of WBS. A total of 36,400 genomic probes spanning a 10 Mb region with 200 bp - 500 bp intervals were selected for the Agilent 4x44K array format. Patients were initially diagnosed with standard Agilent 4x44K arrays and confirmed by FISH. The detailed deletion breakpoint junctions were determined by the high density targeted array CGH study. Five out of six patients showed a deletion of approximately 1,398 Kb with only 100 bp differences at distal and/or proximal breakpoints, indicating non-random recombination between LCRs. One case that showed a ~4,000 Kb deletion from 7q11.22 to 7q11.23 with the standard array displayed two adjacent deletions of 1,693 Kb and 1,390 Kb separated by an 897 Kb undeleted region. The 1,390 Kb deletion corresponds well with the WBS commonly deleted region and shares the same distal breakpoint as in the deletions of the other 5 cases. The 1,696 Kb deletion is centromeric to the common WBS deletion. The deletions in most of our cases occurred between centromeric and medial B block LCRs (Bc and Bm).The detailed mapping of WBS deletion breakpoints has not been possible before the use of the high density targeted arrays and the mapping data is of great significance in exploring the pathogenesis and phenotype/genotype correlations of the disease.

Chondrodysplasia: An example of fixed trait mapping in the domestic dog. *H. G. Parker[1], P. Quignon[1], B. VonHoldt[2], T. Spady[1], D. S. Mosher[1], E. Margulies[3], C. D. Bustamante[4], R. K. Wayne[2], E. A. Ostrander[1]* 1) Cancer Genetics Branch, NHGRI, NIH, Bethesda, MD; 2) Dept of Ecology and Environmental Biology, UCLA, Los Angeles, CA; 3) Genome Technology Branch, NHGRI, NIH, Rockville, MD; 4) Dept of Biological Statistics and Computational Biology, Cornell University, Ithaca, NY.

Dog breeds are isolated populations created through application of strict breeding practices aimed at maintaining a set of characteristics deemed ideal for the breed. While the combination of traits is unique in each breed, the individual traits are not. This population architecture can be used to find the genes responsible for traits that have been bred to fixation in multiple breeds. As a first example of fixed-trait mapping we examined the phenotype of asymmetrical dwarfism or chondrodysplasia. Archetypal chondrodyplastic breeds such as Basset hounds and Dachshunds are characterized by very short legs with normal sized heads and bodies. Many of these breeds share little in common outside of the short-legged phenotype: they fall into different breed clusters in phylogenetic studies, display a variety of coat types, cranial morphologies, and behaviors, and originate from different geographic regions. A search for selected regions in all of these breeds that are not found in proportionate breeds should produce a genetic mutation responsible for chondrodysplasia. To investigate this hypothesis we have run 835 dogs from 75 different breeds including seven chondrodysplastic breeds on the Affymetrix 127K canine SNP chip. After removing SNPs with greater than 10% missing data, 60% heterozygous calls and minor allele frequency of less than 1%, a set of 41635 informative SNPs remained. Analyses from these data identified a single locus spanning 500 Kb associated with chondrodysplasia (p=10-104). Fine mapping using 70 documented SNPs, 170 newly discovered SNPs and indels, and genotypes from additional chondrodysplastic breeds revealed a region of 35 Kb that is homozygous in all chondrodysplastic breeds. Here we discuss the results from this study including the content and significance of a 10 Kb insert in a highly conserved regulatory region.

CAPL: An association test combining nuclear family and case-control data. *R.-H. Chung, R. W. Morris, M. A. Schmidt, E. R. Martin* Miami Inst Human Genomics, Miami, FL.

Family-based and case-control are commonly used designs for complex disease-gene mapping. It has become increasingly important that statistical tests for association be able to accommodate both types of data in a single analysis. We have developed a method, CAPL, for combining family and case-control data based on the Association in the Presence of Linkage (APL) procedure (Martin et al. 2003). Case-control data are integrated into a family-based framework by treating cases and controls as triad families with missing parents. CAPL infers missing parental genotypes based on offspring genotypes, thereby estimating parental genotypes for cases and controls jointly with family data. The APL statistic, which is based on the difference between the observed number of alleles in affected siblings and the expected number of alleles conditional on observed or inferred parental genotypes, is calculated for the combined data. A bootstrap procedure is used to estimate the variance of the APL statistic. We use simulations to demonstrate that CAPL has correct type I error rates under the null hypothesis of no linkage or no association between disease and marker loci. Other association tests that combine nuclear family and case-control data include SCOUT (Epstein et al. 2005) and UNPHASED (Dudbridge 2008). Since CAPL uses families with missing parents and multiple affected siblings, it is more flexible than SCOUT. Using simulated triad family and case-control data, we find that SCOUT can have more power than CAPL and UNPHASED. Under a recessive genetic model, we find that CAPL can have more power than UNPHASED when multiplex families with missing parents and case-control data are combined.

***PIK3CA* mutations are frequent in clinically aggressive forms of endometrial cancer.** *D. Bell[1], M. Rudd[1], S. Fogoros[1], A. Godwin[2], M. Merino[3]* 1) NHGRI/NIH, Bethesda, MD; 2) Fox Chase Cancer Center, Philadelphia, PA; 3) NCI/NIH, Bethesda, MD.

Endometrial cancer is the 8th leading cause of cancer death among women in the United States. Tumors are classified as either type I or type II based on differences in histology, epidemiological risk factors and molecular characteristics. Type I tumors have an endometrioid histology whereas type tumors consist of serous and clear cell histologies. Although type II tumors comprise only 1 in 7 cases at diagnosis they are clinically aggressive and contribute to approximately half of all endometrial cancer related deaths. Currently, the molecular alterations underlying type II tumorigenesis are poorly understood. The *PIK3CA* gene encodes a lipid kinase that activates the AKT signal transduction pathway, which controls cell proliferation and survival. Although *PIK3CA* is one of the most frequently activated oncogenes in human cancer, and represents an attractive therapeutic target, the incidence of *PIK3CA* mutations in type II endometrial tumors has not been defined. We used nucleotide sequencing to search for mutations within all coding exons of *PIK3CA*, and quantitative real-time PCR to evaluate gene copy number, among 69 primary type II endometrial tumors. We detected 27 somatic *PIK3CA* mutations among 20 of 69 (28%) tumors. Six cases had two or more mutations. Most variants (19 of 27, 70%) affected codons that undergo mutation in other tumor types suggesting they are likely to be functionally significant. Increased *PIK3CA* copy number was rare, being detected in a single tumor that had a concomitant *PIK3CA* mutation. In contrast to tumors from other tissue types, in which *PIK3CA* mutations occur predominantly within exons encoding the helical and kinase domains, mutations within our series were dispersed throughout the coding region. In conclusion, our findings indicate that *PIK3CA* is mutated at an appreciable frequency in type II endometrial cancer, and with a unique mutation spectrum. Thus, therapeutic agents that disrupt the PIK3CA-AKT signal transduction pathway may merit future consideration in the clinical management of patients with serous or clear endometrial cancer.

Mapping of Vascular Calcified Plaque Loci on Chromosome 16p in the Diabetes Heart Study. *A. B. Lehtinen[1], V. S. Voruganti[2], M. E. Rudock[1], J. T. Ziegler[1], B. I. Freedman[1], J. J. Carr[1], A. G. Comuzzie[2], C. D. Langefeld[1], D. W. Bowden[1]* 1) Wake Forest Univ Sch of Medicine, Winston-Salem, NC; 2) SW Foundation Biomedical Res, San Antonio, TX.

Cardiovascular Disease(CVD) is the leading cause of death among Americans and is the most common cause of death in type 2 diabetes(T2D).All manifestations of CVD are more common in diabetic than non-diabetic patients.Vascular calcification is a subclinical measure and independent risk factor for CVD.We have reported evidence for a carotid artery calcified plaque(CarCP) locus on chromosome 16p(LOD 4.39 @ 8.4cM) in European Americans(EA) with T2D from the Diabetes Heart Study(DHS).We performed fine-mapping of 16p and evaluated candidate genes and SNPs to ascertain their contribution to the variation in measures of subclinical CVD.69 HapMap SNPs were chosen for fine-mapping and an additional 58 SNPs were genotyped in 6 genes(CACNA1H, SEPX1, ABCA3, IL32, SOCS1, KIAA0350) in the EA DHS subjects consisting of 937 subjects from 315 pedigrees with at least two individuals with T2D.Linkage mapping data were analyzed using SOLAR adjusting for age, gender, BMI, and T2D status.SNP association analysis was also performed using SOLAR with the same covariates.Fine mapping resolved the CarCP linkage peak into 2 distinct linkage regions with maxLOD of 3.89 at 6.9cM and 4.86 at 16.0cM in EA T2D individuals.When all EA subjects were included, the maxLODs were 3.72 at 9.8cM and 2.61 at 16.0cM.Evidence of linkage for coronary calcified plaque(CCP; LOD 2.27 @ 19cM) and a vascular calcification principle component(LOD 3.71 @ 16.0cM) was also observed.The strongest evidence for association with CarCP was observed in and near A2BP1(rs4337300 p=0.005).Furthermore, there was modest evidence of association between CCP and CarCP with SNPs in CACNA1H(p=0.010-0.033) but no single locus explains the evidence for linkage.SNP data was further analyzed using Bayesian Quantitative Trait Nucleotide analysis which identified a SNP, rs1358489, which has either a functional effect on CarCP or is in LD with a functional SNP.This study has substantially refined the 16p region through complementary fine-mapping and candidate gene analysis.

Ordered Subset Analysis for Case-Control Association Mapping (OSACC). *X. Qin, E. Hauser, S. Schmidt* Center for Human Genetics, Duke University Medical Center, Durham, NC.

Genetic heterogeneity can reduce the power for complex disease gene mapping since only a fraction of the cases in the collected dataset may carry a specific disease susceptibility allele. Ordered subset analysis (OSA) was originally designed as a linkage test that identifies a subset of families with the strongest linkage signal based on the ranking of family-specific continuous covariate values. The same strategy can be applied to association analysis to identify a subset of cases that provides the strongest association evidence. The OSACC algorithm adds one or more cases at a time on the basis of their ranked covariate values and re-calculates the contingency table chi-square statistic. The subset of cases that generates the largest chi-square statistic is identified. A permutation procedure is used to approximate the distribution of the maximized chi-square statistics under the null hypothesis that the covariate is uncorrelated with disease risk and marker genotypes; this generates an empirical p-value for evaluating whether the observed association evidence in the subset of cases is greater than expected by random case selection. We performed a comprehensive simulation study under different disease-generating models, including gene-environment interaction, genetic heterogeneity, presence of a quantitative trait locus (QTL) and population stratification. We found that the type I error rate of OSACC can be inflated in the presence of population stratification when only covariate information from cases, not controls, is used. OSACC has higher power to detect a disease susceptibility allele in the presence of extensive genetic heterogeneity, compared to standard tests that either ignore covariates (trend test) or test the relationship between covariate values and marker genotypes directly (ANOVA). We distribute novel software implementing OSACC, in which the user may choose to use covariate data from either cases only, or from cases and controls. In the former case, the software implements a method very similar to the one proposed by Macgregor et al. (2006).

Familial aggregation of common sequence variants on 15q24-25.1 in lung cancer. *P. Liu[1,2], H. Vikis[1,2], D. L. Wang[1,2], Y. Lu[1,2], Y. Wang[1,2], M. You[1,2], Genetic Epidemiology of Lung Cancer Consortium* 1) Dept of Surgery, Washington Univ, St Louis, St Louis, MO; 2) The Alvin J. Siteman Cancer Center, Washington Univ, St Louis, St Louis, MO.

Three recent genome-wide association studies identified association of markers on chromosome 15q24-25.1 with lung cancer risk (1-3). We conducted a genome-wide SNP association analysis in 194 familial lung cases and 219 cancer-free controls and identified several regions of association. Common sequence variants on 15q24-25.1, spanning LOC123688, PSMA4, CHRNA3, CHRNA5 and CHRNB4, were shown to be associated with lung cancer in the current study. The 15q24-25.1 variants appear to contribute to familial aggregation of lung cancer. Risk of lung cancer is increased over five fold among those subjects having family history of lung cancer and taking two copies of risk alleles of the 15q24-25.1 locus.

NPY and ADORA2a Genes, Incidence and Prevalence of Proliferative Diabetic Retinopathy. *B. A. Charles[1], T. J. Orchard[2], S. M. Sereika[3], M. B. Gorin[4], R. E. Ferrell[5], Y. P. Conley[1,5]* 1) University of Pittsburgh, SON, Dept. HP&D, Pittsburgh, PA. 15261; 2) University of Pittsburgh, Dept. Epidemiology GSPH, Pittsburgh, PA 15213; 3) University of Pittsburgh, SON, Dept. H & CS, Pittsburgh, PA 15261; 4) The Jules Stein Eye Institute, David Geffen School of Medicine, UCLA, CA; 5) University of Pittsburgh, Dept. Genetics, GSPH , Pittsburgh, PA 15213.

Diabetic retinopathy (DR) is a microvascular complication of diabetes marked by micro-aneurysms, retinal hemorrhages, exudates; capillary nonperfusion; retinal edema, ischemia and neovascularization. Proliferative diabetic retinopathy(PD)the most severe form of DR is the leading cause of new cases of blindness in individuals between 20 and 70 yrs old, up to 24,000 new cases annually.

Neuropeptide Y (NPY) plays a role in wound healing, diabetes severity, vasoconstriction, and angiogenesis. The Adenosine A2 receptor (ADORA2A) tissue impairment caused by, inflammation, hypoxia, and oxidative stress. Our aim was to determine if the NPY gene or the ADORA2A gene play a role in PD. Two tagging single nucleotide polymorphisms (tSNPs) of the ADORA2A (rs2236624 and rs4822489) and 2 tSNPs of the NPY genes (rs16143 and rs16145) were selected via HapMap. We genotyped (TaqMan allelic discrimination assays) participants (n=496) of the Pittsburgh Epidemiology of Diabetes Complications (EDC) prospective study of childhood onset type 1 diabetes (baseline mean age 28 yrs and mean diabetes duration 19 yrs) for whom banked DNA was available. Stereoscopic images were obtained at baseline (1986-1988) and biennially for 18 yrs. PD was defined as grade 60 in one eye or < 60 but with panretinal scars consistent with laser therapy, according to the Arlie House system.

Univariate analysis showed rs2236624 was associated with prevalence of PD (OR=1.68; 95%CI=1.11-2.54; p=0.03) at baseline and the prospective incidence of PD (HR=0.17; 95%CI=0.042-0.69; p=0.01).

No association was found between the 2 tSNPs of the NPY gene or the rs4822489 tSNP of the ADORA2 gene and prevalence of PD at baseline or prospective incidence of PD.

Fx-1006A: A Novel Small Molecule Stabilizer of Tetrameric Wild-Type and Variant Transthyretin (TTR). *J. Fleming[1], C. E. Bulawa[1], J. Packman[1], R. Labaudiniere[1], T. Coelho[2], J. Kelly[3], L. Wang[4]* 1) FoldRx Pharmaceuticals, Cambridge, MA; 2) FAP Clinical Unit and Neurophysiology Department, Hospital San Antonio, Portugal; 3) Scripps Research Institute; 4) Novartis Institutes for Biomedical Research.

Background: TTR amyloidosis (ATTR) is caused by dissociation of tetrameric TTR (due to genetic mutations or aging) and formation of amyloid deposits. The autosomal dominant disease has >80 known TTR mutations and presents primarily with neuropathy or cardiomyopathy. Fx-1006A, a novel small molecule that binds to the thyroxine binding site of tetrameric TTR, is a potent inhibitor of TTR dissociation, the critical step in TTR amyloid formation. Methods: An immunoturbidimetric assay was developed to determine Fx-1006A stabilization of tetrameric TTR in plasma. Human plasma samples are incubated under denaturing conditions for 48 hours and the fraction of remaining tetrameric TTR is determined. Data is expressed as fold stabilization compared to an untreated control plasma pool. Plasma from healthy volunteers given qd Fx-1006A 15, 30, 60 mg or placebo x 14 days in a Phase I study and V30M ATTR patients given qd Fx-1006A 20 mg or placebo x 8 weeks in a Phase II/III study were assayed. Results: In volunteers (N=24), TTR levels 24 hrs post last dose were 23-42 mg/dL, with stabilization achieved at Fx-1006A concentrations of 0.9-1.2g/mL (15mg) and 1.3-4.2g/mL (30mg), (fold stabilization vs placebo of 2.040.44 and 3.570.92, respectively; $p<0.05$). Saturation of stabilization occurred at or before 30mg, with no significant difference between 30 and 60mg ($p=0.46$). Saturation is observed when Fx-1006A:TTR stoichiometry reaches 1, achieved with Fx-1006A levels of ~1-2g/mL. TTR stabilization was also achieved in V30M ATTR patients at 8 weeks (2.660.7 fold stabilization for treated (n=6) vs placebo (n=4); $p=0.0019$); TTR levels were 20-33mg/dL, Fx-1006A concentrations were 0.7-2.8g/mL. Conclusions: Fx-1006A stabilizes both wild-type and variant TTR in vivo, and should provide disease modification and halt disease progression in ATTR. A Phase II/III study in V30M ATTR patients is ongoing, with final results expected in mid 2009.

Characterization of an ENU-induced mutant mouse uncovers a novel gene, *Thm1 (Ttc21b)*, important for Sonic hedgehog (SHH) signaling and cilial function. *P. V. Tran[1], T. Y. Besschetnova[2], J. V. Shah[2], D. R. Beier[1]* 1) Genetics Division, Brigham and Women's Hospital/Harvard Medical School, Boston, MA; 2) Renal Division, Brigham and Women's Hospital/Harvard Medical School, Boston, MA.

We have recently described the characterization of an ENU-induced mutant mouse, *alien (aln)*, which displays preaxial polydactyly, craniofacial abnormalities and neural tube defects, characteristic of inappropriate activation of SHH signaling (Tran et al., Nature Genetics, 40: 403-10, 2008). Positional cloning revealed a missense mutation in a novel gene, *Thm1* (Tetratricopeptide repeat -containing Hedgehog modulator 1, also called *Ttc21b*), an orthologue of *Flagellar Associated Protein 60/IFT139* in *Chlamydomonas*. Vertebrate cilia, synonymous to flagella of *Chlamydomonas*, are microtubule-based extensions of the plasma membrane in which proteins are transported bidirectionally in intraflagellar transport (IFT). Defects in cilial physiology underlie an increasing number of human diseases or ciliopathies. In *aln* mutants, cilia are shortened and have bulb-like structures at their tips in which IFT proteins are sequestered. RNAi knock-down of *Thm1* in mouse inner medullary collecting duct (IMCD) cells recapitulated the *aln* cilial phenotype and live imaging of IFT88-EYFP in these cells revealed a defect in trafficking proteins away from the cilial tip toward the base. We propose that this retrograde IFT defect causes sequestration of IFT proteins in *aln* cilia and leads to the hyper-activated SHH signaling phenotype. Importantly, *aln* is the first cilial mutant which separates the components of IFT and their roles in SHH signaling. To explore this in more detail, we have adapted the IMCD cells so that SHH signaling can be directly measured in this *in vitro* system. We are using live cell imaging of GFP- and RFP-tagged GLI2 to investigate the processing events that mediate the activation of this transcription factor, which are as yet unknown. Finally, since perturbations of *Wnt* signaling have been reported in cilial mutants that show defective SHH signaling, we are exploring whether the *Wnt* pathway is affected in *aln* mutant cells and mice.

Chromosome abnormalities associated with features of Pervasive Developmental Disorder. *A. D. Rasalam, J. C. Dean* University of Aberdeen, Scotland.

Autistic disorder is a neurodevelopmental disorder charcterised by three core symptom domains: impaired social communication and language, impaired social interaction and repetitive and stereotyped behaviours. Autism spectrum disorders or Pervasive Developmental Disorders have been estimated to occur at a prevalence of approximately 60 per 10,000 school age children. They are associated with speech or general developmental delay and other features such as epilepsy, facial dysmorphology and malformations in a proportion of cases. We report on the chromosome anomalies identified in six patients who also fulfilled the Diagnostic and Statistical Manual of Mental Disorders IV (DSM IV) criteria for diagnosis of Autistic Disorder. The abnormalities identified involved chromosomes 1q, 9q, 11q, 15q, 18p, 18q, 20q and Xp. Three of the anomalies were identified using MLPA analysis and are in subtelomeric regions, two were identified by FISH and one was identified by karyotyping. In five cases, the abnormalities were de novo while in one case it was maternal in origin. All these patients had a degree of developmental delay. Only 2 patients had major malformations. One patient who did not have malformations had poor growth (2nd centile), one was obese and another had growth parameters above the 97th centile. One patient also has epilepsy. These six cases are selected from a larger group of patients with chromosome abnormalities who also have developmental delay and one or more of the features of a pervasive developmental disorder, but who did not fulfil the DSM IV criteria for diagnosis of Autistic Disorder. The features present in this larger group of cases and the chromosome anomalies that were identified in them are also described.

Procedure for detecting genes and environmental factors for common diseases dealing with competing risks and comorbidity. *N. Tanaka[1], M. Muramatsu[2], T. Yamaguchi[3], M. Sawabe[4]* 1) Dept Biostatistics, Harvard Sch of Public Health, Boston, MA; 2) Dept Molecular Epidemiology, Medical Research Institute, Tokyo Medical and Dental Univ, Tokyo, Japan; 3) Dept Clinical Trial Data Management, Grad Sch of Medicine, Univ Tokyo, Tokyo, Japan; 4) Dept Pathology, Tokyo Metropolitan Geriatric Hospital, Tokyo, Japan.

In the previous decade, genetic association studies have generated many data concerning the genetic basis of common multifactorial diseases. Several genetic polymorphisms have been linked to more than one disease, for example, polymorphisms in the ACE, APOE, and TGF- gene, which suggest that there should be competing risk factors for a multifactorial disease. However, we would or could not collect the data on other diseases that could be competing risks when we conduct a case-control study. It has been well established that the proportion of people with multiple diseases may vary from 30% in the general population to over 50% in people aged 60 years and older. If people die with other than a primary disease of interest, the other diseases is defined as competing risks and it is difficult to evaluate true effect of genes on development of the diseases. Co-morbid status would extract useful information about competing risks and genetic risk factors which are shared with some diseases. We present a procedure to investigate whether a specific combination of genes and environmental risk factors results in a specific combination disorders using a consecutive autopsy study data. Considering combination-combination association, we found APOE had no marginal effect on Ischemic Heart Disease (IHD) but was associated with higher risk of co-occurring IHD and Alzheimer's disease (AD) than of developing only AD. Some other potential interaction effects on a specific disease and combination of diseases are presented. Furthermore, we can infer whether the marginal effect from the standard multiple regression models might be biased due to potential competing risks. The results from this study will be useful for designing of whole genome studies for discovering novel susceptible genes as well as of candidate gene studies.

Hypo-functional *SLC26A4* variants associated with nonsyndromic hearing loss and enlargement of the vestibular aqueduct: genotype-phenotype correlation or coincidental polymorphisms? *B. Y. Choi[1], A. Stewart[2], A. Madeo[1], S. Pryor[1], S. Lenhard[1], R. Kittles[3], K. Arnos[4], W. Nance[5], K. King[1], C. Zalewski[1], C. Brewer[1], L. Karniski[6], S. Alper[2], A. Griffith[1]* 1) NIDCD/NIH, Rockville, MD; 2) Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA; 3) University of Chicago, Chicago, IL; 4) Gallaudet University, Washington, DC; 5) Medical College of Virginia, Richmond, VA; 6) Veterans Affairs Medical Center and University of Iowa College of Medicine, Iowa City, IA.

Hearing loss with enlargement of the vestibular aqueduct (EVA) can be associated with mutations of the *SLC26A4* gene encoding pendrin, a transmembrane $Cl^-/I^-/HCO_3^-$ exchanger. Pendrins critical transport substrates are thought to be I^- in the thyroid gland and HCO_3^- in the inner ear. We previously reported that bi-allelic *SLC26A4* mutations are associated with Pendred syndromic EVA whereas one or zero mutant alleles are associated with nonsyndromic EVA. Scott et al. (HMG, 2000) proposed a correlation of nonsyndromic EVA with *SLC26A4* alleles encoding pendrin with residual transport activity. Here we describe the phenotypes and *SLC26A4* genotypes of 47 EVA patients ascertained since our first report of 39 patients. We sought to determine the pathogenic potential of each variant in our full cohort of 86 patients. We evaluated the trafficking of 11 missense pendrin products expressed in COS-7 cells. Products that targeted to the plasma membrane were expressed in Xenopus oocytes for measurement of anion exchange activity. p.F335L, p.C565Y, p.L597S, p.M775T, and p.R776C had Cl^-/I^- and Cl^-/HCO_3^- exchange rate constants that ranged from 13 to 93% of wild type values. p.F335L, p.L597S, p.M775T and p.R776C are typically found as mono-allelic variants in nonsyndromic EVA. The high normal control carrier rate for p.L597S indicates it is a coincidentally detected nonpathogenic variant in this context. We observed moderate differential effects of hypo-functional variants upon exchange of HCO_3^- versus I^- but their magnitude does not support a causal association with nonsyndromic EVA. However, these alleles could be pathogenic in trans configuration with a mutant allele in Pendred syndrome.

Sample Size Calculations in Multiple Matched Case-Control Studies with Binary Exposure Levels. *M. Rao, R. Chakraborty, X. Liu* Ctr Genome Information, Univ Cincinnati, Cincinnati, OH.

The focus of this research is on 1:M matched case-control studies. For each case (the one with the disease under scrutiny), M (1) controls (those who do not have the disease) are chosen from the same population matched on a number of variables such as age, gender, race, etc. A matched set will thus have (M+1) subjects. Each subject is either exposed or not. The goal is to examine association between the disease and exposure. The null hypothesis of interest is that the population odds ratio ; is unity. We propose Raos score statistic for this problem. We work out a sample size formula for a given level, size, and alternative value of ;. The formula involves a nuisance parameter. We compare the performance of out test with the one proposed by Ejigou (Biometrics, 52, 1996). We show that our method is more cost-effective. For example, when M = 2, =1.5, level = 0.05, power = 0.80, nuisance parameter = 0.45, our sample size is 165 and Ejigou's sample size is 174. Simulations with our sample size yields observed power 0.7924 and with Ejigou's sample size 0.8195. The difference between the sample sizes is much more pronounced with other choices of the inputs with our sample size being lower.

A novel genomic (CGH) microarray abnormality in a patient with clinical features of KBG syndrome - a case report. *S. Ahmed, K. Wendt, C. Spring, M. Jamehdor* Regional Genetic Service, SCPMG, Kaiser Permanente.

INTRODUCTION: Comparative genomic hybridization (CGH) has become a commonly ordered test in Medical Genetics practice. However, the interpretation of abnormal CGH results is complicated by the limited number of mutation databases providing genotype phenotype correlation. We report a case of an 11 yr old girl with an abnormal CGH microarray result and clinical features of KBG syndrome, a clinical syndrome characterized by macrodontia, costovertebral anomalies and mental retardation. The genetic basis of KBG syndrome is currently unknown. CASE REPORT: Our patient is an 11 yr old girl referred to genetics clinic for evaluation of her dysmorphic facial features, behavioral problems and severe delays. Pregnancy history was essentially unremarkable. Dysmorphic facial features and hypotonia were noted at birth. During childhood, short stature, severe developmental delay and self-mutilating behavioral abnormalities including hand-biting and head-banging were noted.Dysmorphic features include mild hypertelorism, a large mouth with large prominent incisors and short stubby hands. Family history was unremarkable. Brain MRI, chromosome analysis and FISH for Smith Magenis were negative. Imaging showed delayed bone age.A clinical diagnosis of probable KBG syndrome was made. CGH microarray demonstrated a deletion on chromosome 4 involving 2 BAC clones within the 4q21.21 region, suggesting partial monosomy for this region of chromosome 4. FISH using a BAC within the abnormal region confirmed the microarray findings. Mother tested negative for the deletion. The patients father is deceased. Close paternal relatives could not be tested. DISCUSSION: With the increasing use of CGH, the novel genetic changes that are being reported may elucidate the molecular etiology of various clinical dysmorphology syndromes. Our patient has some features of KBG syndrome. Self-mutilation has not been described with this syndrome. Whether or not our patient has a variation of KBG syndrome, or another distinct clinical syndrome, remains unclear. Individual case reports may help define these CGH abnormalities and aid in clinical correlation.

E297K Mutation of Calcium-sensing Receptor (CASR) Gene is Associated with Short Stature and Impaired Growth Hormone Response. *W. Chen[1], A. Bos[2], L. Sloper[2], E. R. Miller[2], S. Basaria[2], N. B. McDonnell[1,2]* 1) Lab Clinical Investigation, NIA/NIH, Baltimore, MD; 2) Clinical Research Branch, NIA/NIH, Baltimore, MD.

The calcium-sensing receptor (CASR) is a plasma membrane G protein-coupled receptor that is expressed in the parathyroid hormone (PTH)-producing chief cells of the parathyroid gland and the cells lining the kidney tubule. By virtue of its ability to sense small changes in circulating calcium concentration and to couple this information to intracellular signaling pathways that modify PTH secretion or renal cation handling, CASR plays an essential role in maintaining mineral ion homeostasis. Mutations in the CASR gene have resulted in different human diseases. E297K, a loss of function mutation in the CASR, is associated with familial hypocalciuric hypercalcemia (FHH) and neonatal severe hyperparathyroidism (NSHPT). Here we report a dominant E297K mutation that is transmitted in a three-generation Brasilian family with short stature and variable hypercalcemia. The arginine/growth hormone challenge test in the proband, an adult male with short stature, showed an impaired growth hormone response, despite normal serum IGF-1 level. Prior studies have shown that in patients with primary hyperparathyroidism, growth hormone response can be blunted similar to our patients. Correction of serum calcium in these patients resulted in normalization of growth hormone secretion. We propose that elevated serum calcium is responsible for the short stature and blunted growth hormone secretion in our patients. FHH may not be as benign as previously thought and should be considered in the differential diagnosis of short stature. Familial short stature may in fact be familial hypocalciuric hypercalcemia in some cases.

High-resolution analysis of subtelomeric breakpoints. *J. Jackson, K. Rudd* Department of Human Genetics, Emory University School of Medicine, Atlanta, GA.

Between 3-6% of patients with idiopathic mental retardation have a detectable subtelomeric rearrangement, a genomic change in the end of the chromosome. Subtelomeric rearrangements occur on all chromosome ends, and are a major cause of birth defects, mental retardation, and developmental delay. Despite the frequency of double-strand breaks at chromosome ends, the mechanisms of subtelomeric breakage and repair remain unknown. To elucidate this process, we have performed the first high-resolution analysis of subtelomeric breaks via a combination of high-density array CGH, breakpoint cloning, and comprehensive sequence analysis. Using a custom oligonucleotide array, we have mapped 18 subtelomeric rearrangements at a resolution previously unattainable. We have analyzed 12 terminal deletions, 2 terminal duplications, and 4 unbalanced translocations, resolving breakpoints to a few hundred basepairs at each site. To isolate the repaired genomic structure and identify sites of double-strand breaks we are cloning breakpoint junctions. We have cloned one junction created by an unbalanced translocation between the X chromosome and chromosome 16. Breakpoint cloning and sequence analysis of breakpoint regions at other sites is ongoing. We postulate that subtelomeric sequences are predisposed to breakage, as subtelomeres are enriched in certain types of repeats known to cause genomic instability at other loci. Subtelomeres are incredibly variable and a major source of polymorphism in the human genome. We predict that particular subtelomeric sequence variants are more likely to rearrange in meiosis, thereby affecting the risk of having a child with a subtelomeric rearrangement. Based on the prevalence of subtelomeric rearrangements, the enrichment of subtelomeric breaks in mitosis and meiosis, and the repetitive sequences at subtelomeres, we hypothesize that subtelomeres are particularly susceptible to breakage. We present our initial results detailing the mechanisms of human subtelomeric breaks, with broader implications for the factors mediating other chromosome rearrangements.

Identification of a Novel Chromosome 11 Locus from Murine Strain 129 that Protects from Sex Reversal in the B6 XYPOS Mice. *V. Arboleda, E. Vilain* UCLA Department of Human Genetics.

Disorders of sexual development (DSD) encompass a vast spectrum of phenotypes ranging from minor malformations of genitalia (hypospadias cryporchidism, clitoral hypertrophy) to sexual ambiguity. Together, these anomalies have a frequency estimated at 0.5 % to 1% of live births. Despite the prevalence of these malformations, the genetic etiology a majority of DSDs remain unexplained. Mouse models of disorders of sex development are invaluable. The presence of the Mus domesticus poschiavinus Y chromosome, YPOS on the C57Bl/6J (B6) genetic background results in the development of ovarian tissue (i.e. sex reversal) in all genotypic XY animals. The same YPOS chromosome on the 129S1/SviMJ (129) background results only in the development of testicular tissue in XY mice. We have previously reported the existence of a congenic region of 129 origin, on chromosome 11, conferring protection from XY sex reversal. In nineteen B6-XYPOS animals, investigation of the gonads (testis, ovary or ovotestis) revealed 10 XY-females, 7 XY-males, and 2 hermaphrodites, consistent with susceptibility to XY sex reversal. However, in the B6.129-YPOS congenic animals, of 20 animals analyzed, 18 XY-males, 2 XY-females, and no hermaphrodites were identified, showing significant protection against sex reversal. In addition, we generated, by backcrossing to B6, a line carrying a subset of the congenic region. 15 genotypic males homozygous for a subcongenic fragment between position 0 and 20Mb on chromosome 11 were analyzed at 4-6 weeks of age. 8 XY mice had 2 ovaries, 1 was classified as a hermaphrodite, with both an ovary and a testis, and 6 were phenotypic males with two testes. These results suggest protection from XY sex reversal by the whole congenic region, but absence of protection by the 0-20Mb subcongenic region. Marker analysis localizes the congenic fragment protecting against XY sex reversal between position 60Mb and 95Mb on chromosome 11. This new locus contains several genes expressed in the developing gonads that are proposed to account for the sex-reversal phenotype in the B6-YPOS as well as the unexplained genetic etiology of the majority of XY DSDs.

The mitochondrial DNA landscape of modern Mexico. *A. Achilli[1,2], U. A. Perego[2,3], J. E. Gomez-Palmieri[3], R. M. Cerda-Flores[4], K. H. Ritchie[3], A. Pollock[3], N. Angerhofer[3], A. Escobar-Mesa[5], A. Torroni[2], N. M. Myres[3], S. R. Woodward[3], Sorenson Molecular Genealogy Foundation, SLC, Utah (USA)* 1) Dip. di Biologia Cellulare e Ambientale, Universita' di Perugia, Perugia, Italy; 2) Dip. di Genetica e Microbiologia, Università di Pavia, Pavia, Italy; 3) Sorenson Molecular Genealogy Foundation, Salt Lake City, Utah, USA; 4) Genetics Division, Northeast Biomedical Research Center, Instituto Mexicano del Seguro Social, Monterrey, Nuevo Leon, Mexico; 5) Secretaria de Salud, Veracruz, Mexico.

With more than 180 ethnic and linguistics groups, Mexico is a rich source for anthropological and population studies. This country witnessed the rise and fall of major civilizations, including the well-known Maya and Aztec civilizations, but as a result of heavy European colonization and influx, the population landscape has dramatically changed over the past five centuries. Today less than 30% of modern Mexicans identify themselves as being fully or partly Amerindians and the remaining population seems to have very little in common with their pre-Columbian ancestors. However, this is not the case when the maternal genetic component is evaluated in detail. Analysis of the mitochondrial DNA (mtDNA) control region sequences, including HVS-I, HVS-II and HVS-III, from more than 2,000 subjects revealed an overwhelming Native American legacy in the modern Mexican population, with ~90% of mtDNAs belonging to the four major pan-American haplogroups A2, B2, C1 and D1. This finding supports a European contribution to the Mexican gene pool primarily by male settlers and confirms the effectiveness of employing the uniparentally-transmitted mtDNA as a tool to reconstruct a countrys history.

A genome-wide association study identifies new susceptibility variants for male pattern baldness on chromosome 20. *F. F. Brockschmidt[1], A. M. Hillmer[1], S. Hanneken[2], S. Eigelshoven[2], M. Steffens[3], A. Flaquer[3], S. Herms[1], T. Becker[3], D. R. Nyholt[4], Z. Z. Zhao[4], N. G. Martin[4], T. W. Mühleisen[1], S. Moebus[5], M. Bröcker-Preuss[6], R. Erbel[7], R. C. Betz[8], M. P. Baur[3], T. F. Wienker[3], R. Kruse[2], M. M. Nöthen[1,8]* 1) Genomics, Life & Brain Center, University of Bonn, Bonn, Germany; 2) Department of Dermatology, University of Düsseldorf, Düsseldorf, Germany; 3) Institute for Medical Biometry, Informatics and Epidemiology, University of Bonn, Bonn, Germany; 4) Genetic Epidemiology, Queensland Institute of Medical Research, Brisbane, Australia; 5) Institute for Medical Informatics, Biometry and Epidemiology, University Duisburg-Essen, Germany; 6) Clinic of Endocrinology, Central Laboratory Unit Research and Education, University Hospital of Essen, University Duisburg-Essen; 7) Clinic of Cardiology, West-German Heart Center Essen, University Duisburg-Essen, Germany; 8) Institute of Human Genetics, University of Bonn, Bonn, Germany.

We performed a genome-wide association study in 296 individuals with male pattern baldness (androgenetic alopecia) and 347 controls, all of German descent. We found highly significant association at the previously described androgen receptor locus. The best SNP located outside this locus was on chromosome 20 with P=1.3x10-7 (OR=1.90, CI 1.50-2.41). We then investigated the 30 best autosomal SNPs in an independent replication sample of 319 affected and 234 unaffected German individuals and found highly significant association for five SNPs at the chromosome 20 locus. No interaction was detected with the X-chromosomal androgen receptor locus suggesting a role of the chromosome 20 locus in a yet to be identified androgen independent pathway.

CAG repeat instability and gender of the transmitting parent in Huntington disease: a large maternally-derived expansion. *E. M. Ramos[1], J. Cerqueira[2], C. Lemos[1,2], J. Pinto-Basto[1,2], I. Alonso[1.2], J. Sequeiros[1,2,3]* 1) UnIGENe, IBMC, Porto, Porto, Portugal; 2) CGPP, IBMC, Univ. Porto, Portugal; 3) ICBAS, Univ. Porto, Portugal.

Huntington disease (HD) is an autosomal dominant disorder of adulthood, characterized by motor impairment, chorea, behavioural symptoms and dementia. It is caused by the expansion of an unstable $(CAG)_n$, in the first exon of the HD gene, on 4p16.3, resulting in an expanded polyglutamine tract in huntingtin. Alleles below 35 CAGs produce no symptoms, those with 35-39 repeats show incomplete penetrance, and those with 40 CAGs or more are fully penetrant. The CAG repeat is unstable when transmitted to offspring. We now assessed meiotic instability in 53 paternal and 50 maternal transmissions (79 parents). Instability occurred in 71/103 transmissions (69%), and was more frequent when the transmitting parent was the father (t_{101}=2.485, p=0.015). Mean change was +2.11 CAGs for all male (-2 to +30) and +0.02 CAGs for all female transmissions (-5 to 20). Male and female transmissions differed also regarding the proportion of expansions and contractions (X^2_2=11.077, p=0.004). More expansions and fewer contractions occurred in male transmissions, whereas the opposite was observed with transmitting mothers. Interestingly, a patient with onset at age 8 years inherited a large expansion from her mother (who had symptoms at age 18). The mother had a 75 CAGs allele, whereas her daughter had ~95 CAGs. This large expansion through the maternal lineage is uncommon and increased the mean change for female transmissions (excluding it, the range would be -5 to 4 CAGs, and the mean -0.39). There was no difference with gender of the offspring: sons and daughters had a similar proportion of expansions and contractions, either when the CAG repeat was transmitted from the father (X^2_2=0.807) or the mother (X^2_2=0.954). In conclusion, repeat-length changes were dependent on the gender of the transmitting parent, but not on that of the offspring. Of note, was one maternally inherited infantile-onset case; this was due to the additional expansion of about 20 repeats, of an already large repeat in a mother with juvenile-onset.

Development and characterization of Fmr1 and Fmr2 double knockout mice. *Y. Gu, R. Paylor, D. Nelson* Dept Molec & Human Genetics, Baylor Col Medicine, Houston, TX.

X-linked mental retardation is a common inherited disorder and mutations in over 20 genes have been identified to date. Of these, FMR1 is the most common single gene causing mental retardation and is responsible for Fragile X syndrome. Some 600 kb distal to FMR1 lies another gene that can cause mental retardation (MR) or FRAXE disease when mutated: FMR2. A small number of patients have been found to carry deletions of the region spanning FMR1 to FMR2; these individuals have much more severe MR when compared to patients with Fragile X syndrome or FRAXE disease. We created a mouse model carrying both Fmr1 and Fmr2 mutations through meiotic recombination between previously characterized knockout alleles at Fmr1 and Fmr2. This model carries mutations at both Fmr1 and Fmr2 on the same X chromosome, but importantly, has no other mutations for genes in the vicinity. Thus, it will provide the opportunity to explore whether FMR1 and FMR2 have synergistic effects in the cognitive problems seen in humans lacking both genes or whether other genes that may also be deleted in patients play a significant role. Fmr1/Fmr2 double knockout mice were characterized for general and behavioral phenotypes. They showed reduced brain weight, especially in the cerebra, which was not observed in either Fmr1 or Fmr2 single knockout mice. The increased weight of testis (macroorchidism) resulting from Fmr1 mutation was also observed. Behavioral tests showed additional abnormalities, including a defect in the Rotarod test of motor learning in double knockout mice, while both Fmr1 and Fmr2 single knockout models were unchanged from wild type animals. These results demonstrate for the first time that Fmr1 and Fmr2 play synergistic roles in neural development and function. Ongoing efforts to characterize this model for finer scale neuro-anatomy and for electrophysiology will allow improved understanding of the interaction between these genes. Molecular analysis to determine the mechanisms of interaction will also provide insight into the functions of each of these X-linked MR genes, and may offer insight into the severe MR found in patients lacking both FMR1 and FMR2.

The Human Phenotype Ontology. *P. N. Robinson, S. Köhler, S. Bauer, D. Seelow, D. Horn, S. Mundlos* Institute for Medical Genetics, Charité-Universitätsmedizin, Berlin, Germany.

There are many thousands of hereditary diseases in humans, each of which has a specific combination of phenotypic features. Although humans are particularly good at recognizing human phenotypic anomalies, research employing a systematic approach to the relationships of phenotypes to human cellular biology is still in its infancy. A number of considerations suggest that an ontological description of human phenotypes has distinct advantages over hierarchical systems, including the ability to relate concepts (terms) to multiple parents, to allow descriptions and queries at different levels of granularity and completeness, and also to take advantage of algorithms that have been developed for ontological analysis following the success of the Gene Ontology. These considerations prompted us to develop an ontology with currently over 8000 terms to describe human phenotypic abnormalities. Each term in the HPO describes a phenotypic abnormality such as Atrial septum defect. Terms are related to parent terms by is a relationships. We used an ontological similarity measure between the diseases listed in OMIM in order to visualize and cluster the human phenome. We compared the observed clustering to 21 predefined physiological disorder classes to clusterings obtained from 10,000 networks in which edges were randomly rewired 2,000 times. The actual network score was 47.2 standard deviations above the mean random score. We will demonstrate that searches for clinical diagnoses using the HPO achieve a high degree of accuracy that is not overly sensitive to noise, completeness, or specificity of the set of phenotypic terms used for the search. We will demonstrate the use of the HPO to generalize our method for prioritizing candidate disease genes from disease families using random walk analysis (Köhler et al., Am J Hum Genet, 2008). The HPO is freely available under an open-source license, and we are actively seeking the involvement of the community to refine and extend the ontology and disease annotations. Downloads and project information are available at the project hompeage, http://www.human-phenotype-ontology.org.

Functional analysis of the rheumatoid arthritis associated 6q23 region; regulation of TNFAIP3. *L. Elsby[1], T. Eastell[1], J. Worthington[1], D. Ray[2], R. Donn[1]* 1) arc Epidemiology Unit, University of Manchester, Manchester, United Kingdom, M13 9PT; 2) Centre for Molecular Medicine, Faculty of Medicine, University of manchester, Manchester, United Kingdom, M13 9PT.

Recently, two independent studies have shown association of the 6q23 region with rheumatoid arthritis (RA). The WTCCC genome wide association study of RA found association with a SNP in the 6q23 region (rs6920220); this has since been replicated in a cohort of 5063 UK RA cases and 3849 controls. This SNP lies in an intergenic region between Oligodendrocyte Lineage Transcription Factor 3 (OLIG3) and Tumour Necrosis Factor Alpha-Induced Protein 3 (TNFAIP3) and is in complete LD (r^2=1) with 4 other SNPs (rs6933404, rs2327832, rs6927172 & rs17264332). TNFAIP3 is a candidate gene for investigation in RA as it is a negative regulator of TNFa signalling. To determine the transcriptional regulatory capacity of the 6q23 SNPs on TNFAIP3, 1Kb DNA sequences encompassing the SNPs were cloned into the reporter plasmid pGL3 in both the sense and antisense orientation, with the TNFAIP3 promoter driving reporter gene expression. Site directed mutagenesis was used to alter the SNP alleles. Transfection of the constructs into a T lymphoblast cell line, CEMC7A, demonstrated three of the five SNP sequences (rs 6920220, rs6933404 & rs6927172) were able to repress the activity of the TNFAIP3 promoter in an orientation independent manner, suggesting that these sequences are capable of binding transcription factors that can regulate TNFAIP3 gene expression. The rs6927172 SNP lies in a highly conserved region of homology. Bioinformatic analysis of this sequence showed that the C allele is a consensus ets-1 binding site, and that the G allele disrupts this. Examination of the rs6927172 SNP by electrophoretic mobility shift assay has shown differential protein binding to the allele variants. Further studies will aim to characterise this transcription factor binding and also to determine transcription factor binding to the rs6933404 and rs6920220 SNPs. This data represents novel evaluation of the 6q23 intergenic SNPs associated with RA and suggests a relevant role of altered TNFAIP3 expression with RA pathogenesis.

The shared genetic architecture of common autoimmune diseases. *C. Cotsapas[1,2,3], M. Daly[1,2,3], Network of Consortia, Federation of Clinical Immunology Societies* 1) CHGR, Massachusetts General Hospital, Boston, MA; 2) Dept of Medicine, Harvard Medical School, Boston MA; 3) Broad Institute of MIT and Harvard, Cambridge MA.

There is abundant evidence that complex autoimmune/inflammatory (AI) diseases may have overlapping etiologies, shown both by the aggregation of these disorders within families and by the efficacy of common therapies. Genetic evidence such as the association of the PTPN22 R620W polymorphism to type I diabetes, SLE, and rheumatoid arthritis, suggests that this is at least partly due to shared genetic susceptibility to autoimmunity and inflammation. Recent genome-wide association (GWA) studies for several AI diseases have implicated tens of loci in at least one such disorder, with some of these loci harboring associations to more than one disease. We therefore investigated the commonality of susceptibility architecture by conducting a meta-analysis of selected loci in approximately 14,000 cases and 13,000 controls genotyped in GWA studies of six diseases. We have assembled a list of 51 non-MHC loci unequivocally associated to at least one of ankylosing spondylitis, coeliac disease, crohn's disease, multiple sclerosis, psoriasis, rheumatoid arthritis, systemic lupus erythematosus and type I diabetes. Our inclusion criteria are that association in a genome-wide scan and replicated in independent samples at a cumulative significance level of $P < 5e-8$. We have meta-analyzed data for these loci, excluding the ascertainment disease. We find that >25% of loci may be implicated in multiple diseases: for example, 9/11 type 1 diabetes loci are shared with at least one other disease. We also find evidence that some loci may have opposite effects: the PTPN22 R620W polymorphism, for example, appears protective for Crohn's disease. We will replicate these and other discoveries in independent sample collections. We believe that these results will help to differentiate between common mechanisms of autoimmunity and disease- or tissue-specific factors. This research should lead to greater mechanistic understanding of the underlying processes and ultimately to better treatments and therapies.

Life threatening menorrhagia in patients with Glycogen Storage Disease Type I- An unrecognized complication?

S. Austin, P. Kishnani, A. James, M. Spencer-Manzon Duke University Medical Center, Durham, NC.

Type I Glycogen Storage Disease (Glucose-6-Phosphatase or Translocase Deficiency, Von Gierke Disease) is caused by a defect in the glucose-6-phosphate system. The biochemical defect results in hypoglycemia, hyperlipidemia, hyperuricemia, and a prolonged bleeding time. The prolonged bleeding time is due to a von Willebrand disease like defect which has been described as an acquired platelet abnormality related to chronic hypoglycemia. Recurrent/severe epistaxis is often reported. As patients age, complications such as gout, pancreatitis, hepatic adenomas with risk for progression to hepatocellular carcinoma, pulmonary hypertension, osteoporosis, and renal disease are being recognized. Menorrhagia, defined as greater than 80mL of blood loss per cycle, appears to be a problem in reproductive aged females with GSD I. In our cohort of 15 reproductive aged females (ages 12-46 years), five (33%) developed menorrhagia. Initially, four required hospitalization and blood transfusion and one required hormonal treatment. All five were in inadequate metabolic control. The menorrhagia has subsequently been managed by a gynecologist with experience in GSD I. Interventions included hormonal and non-hormonal treatments: endometrial ablation in conjunction with Micronor (norethindrone) as well as Provera (medroxyprogesterone acetate). Alternatives to oral hormonal treatment have included the Mirena intrauterine system, endometrial ablation, and hysterectomy. Before treatment, the gynecologist discussed these options with the treating team. Neutropenia and risk for infection should be considered in GSD Ib. Additional risk factors for menorrhagia, such as anovulation (as a result of thyroid disease or polycystic ovarian disease) and exogenous hormones were also present in some of the patients. Other risk factors for menorrhagia, such as fibroid polyps, endometrial hyperplasia, and cancer, were not noted in any of the patients. This case series will describe each patients course related to the treatment of menorrhagia. This newly recognized complication should be considered when reviewing the history of patients with GSD I and in the management of these cases.

Autistic features with speech delay in a girl with an ~1.5 Mb deletion in 6q16.1, including *GPR63* and *FUT9*. *E. Bocian[1], K. Derwinska[1,2], J. Bernaciak[1], E. Obersztyn[1], P. Stankiewicz[1,2]* 1) Dept. of Medical Genetics, Institute of Mother and Child, Warsaw, Poland; 2) Dept. of Molecular & Human Genetics, Baylor College of Medicine, Houston TX, USA.

Recent studies have shown that up to 40% of the apparently balanced reciprocal chromosome translocations in patients with abnormal phenotype can be accompanied by a chromosome imbalance. We present a 10-year-old girl with mild mental retardation, language delay, and autistic behavior. She had no dysmorphic features and her brain MRI was normal. Karyotype analysis revealed a de novo apparently balanced translocation t(6;14)(q16;q22). Interestingly, autism, schizophrenia, and bipolar disorder have been linked previously to chromosome 6q16q21. Whole genome array CGH analysis with ~385,000 oligonucleotide probes (NimbleGen) identified an ~1.5 Mb deletion in 6q16.1. FISH with BAC clones mapping within and directly flanking the deleted segment showed that the deletion arose at the translocation breakpoint. The deleted segment harbors *FUT9, GPR63, FHL5, KLHL32, c6orf66*, and *AK091365*, but does not involve *GRIK2*, previously linked and associated with autism and schizophrenia. *GPR63* (*G-protein-coupled receptor 63*) is expressed in the frontal cortex, with lower levels in the thalamus, caudate, hypothalamus and midbrain and encodes a G-protein-coupled receptor for sphingosine 1-phosphate. The *fucosyltransferase 9* (*FUT9*) gene, is highly conserved among humans, mice, rats, and hamsters and is strongly expressed in brain during embryogenesis. *FUT9* is considered to be involved in cell-cell interactions, differentiation, and neurodevelopmental processes. We propose that haploinsufficiency of *GPR63* or *FUT9* may be responsible for the austistic spectrum features present in our patient. Our data confirm previous observations that copy-number variation is a significant factor responsible for autistic spectrum behavior and speech delay.

Familial Aneurysm Syndrome Associated With COL5A1 Mutation. *H. Zhang[1], J. Yang[2], W. Chen[2], F. Cancellario[3], G. Maritati[3], N. McDonnell[1]* 1) CRB, NIA/NIH, Baltimore, MD; 2) LCI, NIA/NIH, Baltimore, MD; 3) San Camillo Hospital, Rome.

We examine here a North American family with a strong history of arterial aneurysms, dissections, and ruptures associated with a premature termination codon in procollagen V(1). The proband, a 32 year old woman, presented features consistent with the classical form of EDS such as joint hypermobility, epicanthal folds, and abnormal scars; however, family history revealed that her father died of a thoracic aorta rupture at age 48 and a paternal aunt developed a 4.1 cm renal aneurysm in her 50s. The probands brother, born with a club foot, was also hypermobile. Protein analysis of procollagen III and I did not reveal any abnormalities. Research based sequence analysis of COL3A1, TGFBR1, and TGFBR2 genes revealed neither pathological mutations nor SNP homozygosity suggesting a deletion.

Imaging showed that the patient had a 1.8 cm ectatic left iliac artery with no perceived immediate risk of rupture. Aggressive blood pressure control was initiated. Two months later, while on vacation in Italy, the patient experienced severe lower quadrant pain and lost consciousness. Rupture of the left iliac was diagnosed, and an emergent repair with Dacron bypass from the right common iliac to her left external iliac artery was performed. Approximately one year later, a new 2.4 cm aneurysm in the right common iliac artery was detected via MRI.

The patients brother with a similar phenotype underwent research imaging studies, and ectasia of the celiac and mesenteric axes were identified.

Due to the phenotypes similarity to classical EDS, a research based sequence analysis of the COL5A1 gene was performed. A premature termination codon in exon 3 was identified in the proband, her brother, and her aunt. Haploinsufficiency mutations in COL5A1 have been previously associated with the classical and hypermobile forms of EDS, but aggressive vascular complications as seen in this family have not been described. COL5A1 mutations should be considered in families with strong histories of vascular complications and features suggestive of classical EDS.

SNP detection algorithm for next generation sequencing technologies. *S. Gopalakrishnan, Z. S. Qin* Biostatistics, University of Michigan, Ann Arbor, MI.

As we move forward with next generation sequencing technologies, we have the opportunity to identify low frequency mutations within each individuals including de novo ones that cannot be detected using SNP panels designed based on common mutations. To avoid ambiguity, most of the current methods only utilize uniquely mapped reads to identify polymorphic loci. This is undesirable since mapping ambiguity may be caused by polymorphisms. In this work we present a Bayesian approach to identify polymorphic loci using short reads including those that are not uniquely mappable. Our method attempts to align the ambiguous reads to their true genomic locations and performs polymorphism detection at the same time. This is achieved by reconciling among observed read sequences, reference genome and the possibility of polymorphic sites. Simulation studies using real sequence trace data showed favorable performance in terms of sensitivity and specificity compared to the method using only uniquely mapped reads. We tried our method on a real dataset generated from Illumina Genome Analyzer. The reads were generated using a 1.8 Mb region on the q arm of chromosome 7 near the CFTR gene. Using one million reads mappings covering a 300 kb region, our method identifies 94 previously known SNP loci. In contrast, using only uniquely mapped reads identifies only 59 previously known sites. Upon increasing the number of mappings to 5 million, covering a 900 kb region, the numbers increases to 189 and 152 respectively.

The XLMR gene *ACSL4* plays a role in dendritic spine architecture. *I. Meloni[1], V. Parri[1], R. De Filippis[1], F. Ariani[1], R. Artuso[1], M. Bruttini[1], E. Katzaki[1], I. Longo[1], F. Mari[1], C. G. Dotti[2,3], A. Renieri[1]* 1) Department of Molecular Biology, Medical Genetics, University of Siena, Siena, Italy; 2) Department of Human Genetics, University of Leuven Medical School, Leuven, Belgium; 3) Department of Molecular and Developmental Genetics, Flanders Institute of Biotechnology (VIB 11) Leuven, Belgium.

ACSL4 is a gene involved in non-syndromic X-linked mental retardation. It encodes for a ubiquitous protein that adds Coenzyme-A to long-chain fatty acids, with a high substrate preference for arachidonic acid; it presents a brain-specific isoform deriving from alternative splicing and containing 41 additional N-terminal aminoacids. To start unravel the link between *ACSL4* and mental retardation, we have characterized the protein and analyzed the consequences of its absence in rat primary hippocampal neurons. By RT-PCR analyses we identified a new transcript with a shorter 5-UTR region and showed that *ACSL4* is expressed at higher levels in fetal than in adult brain. Differential expression of the alternative transcripts in different adult brain regions was also observed. By immunofluorescence microscopy we report that ACSL4 is located preferentially in endoplasmic reticulum. To characterize ACSL4 function in rat hippocampal neurons we silenced the gene by siRNA technology. These experiments suggest that ACSL4 might be dispensable for neurites formation and final length but it is required for the presence of normal dendritic spines. In fact, reduced ACSL4 levels led to a significant reduction in dendritic spine density and an alteration in spine/filopodia ratio and in spine distribution among different morphological categories (stubby, thin and mushroom spines). It has been reported that arachidonic acid, ACSL4 substrate, is involved in the regulation of actin cytoskeleton. These data suggest that ACSL4 might directly or indirectly influence actin cytoskeleton organization; the observed spine anomalies might thus be a secondary effect of an abnormal actin organization due to ACSL4 absence.

Quantifying and correcting for the winner's curse in genome-wide association studies for quantitative traits. *R. Xiao, M. Boehnke* Dept Biostatistics, Univ Michigan, Ann Arbor, MI.

Genetic association mapping is a powerful method to detect genetic variants that influence quantitative traits. As a consequence of winners curse, initial estimates of genetic effect sizes for quantitative trait loci (QTL) tend to be upwardly biased. In this paper, we parameterize the genetic effect of the QTL as the linear regression coefficient of the genotype. In the context of genome-wide association studies or large-scale candidate-gene studies, we analytically quantify the impact of the winners curse on the uncorrected estimator of QTL effect size under different genetic models. We then propose an ascertainment-corrected maximum likelihood method to improve the QTL effect estimate. Simulation results indicate that the proposed method reduces the overestimation by different degrees, depending on study power, consistent with findings from us and others for dichotomous traits.

Novel genetic associations of the antioxidative enzyme glutathione peroxidase 1 with low total bone mineral density in postmenopausal women. *S. Jurkovic[1], J. Osredkar[1], J. Prezelj[2], J. Marc[3]* 1) KIKKB, University Medical Centre Ljubljana, Njegoseva 4, Ljubljana, Slovenia; 2) Department of Endocrinology, Diabetes and Metabolic Diseases, University Medical Centre Ljubljana, Slovenia; 3) Department for Clinical Biochemistry, Faculty of Pharmacy, Ljubljana, Slovenia.

Osteoporosis, a systemic bone disease with low bone mass, affects every second women at the age of 65 years or later. Recently, oxidative stress has been suggested to participate in the development of osteoporosis. Osteoblasts can produce antioxidants such as glutathione peroxidase 1 (GPX1) to protect against reactive oxygen species. The aim of this study was to determine whether the polymorphisms in GPX1 are associated with low bone mineral density (BMD), 1-year BMD changes and biochemical bone turnover markers, such as sBALP, RANKL, ALP, pOC, sOPG and DPyr. The study included 535 postmenopausal women, aged 45-92 years. Each patient was examined for BMD and biochemical bone turnover markers. After a year, BMD was measured again in 160 of the postmenopausal women involved in the study. All subjects were genotyped for polyalanine repeats in the first exon and Pro198Leu variation in the second exon of the GPX1 gene using the non-denaturing DHPLC and RFLP, respectively. The frequencies of genotypes in the control group of 190 subjects were as follows: 5/5 (24.4%), 5/6 (30.7%), 5/7 (18.2%), 6/6 (6.3%), 6/7 (14.2%), 7/7 (6.3%) for the polyalanine repeats and CC (51.1%), CT (44.1%), TT (4.8%) for the Pro198Leu polymorphism. Significant associations between both GPX1 polymorphisms and total BMD values in postmenopausal osteoporotic women were found (p0.005). The genotype 6/6 is strongly associated with a lower total BMD value than the 5/7 genotype (p=0.003). A similar decrease of total BMD was observed in genotypes TT in comparison with the genotype CC (p=0.026). Moreover, no significant associations with the biochemical bone turnover markers were determined. The results show for the first time significant associations between polymorphisms of the GPX1 gene and BMD values and thus the connection of the antioxidative genetic polymorphisms with the osteoporosis.

Extreme short stature resulting from mutation of the C-type lectin domain of aggrecan. *S. W. Tompson[1], B. Merriman[2], V. A. Funari[1], M. Fresquet[3], D. L. Rimoin[1], R. S. Lachman[1], S. F. Nelson[2], M. D. Briggs[3], D. H. Cohn[1,2], D. Krakow[1,2]* 1) Medical Genetics Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA; 2) Human Genetics Department, University of California at Los Angeles, Los Angeles, CA, USA; 3) Wellcome Trust Centre for Cell-Matrix Research, University of Manchester, Manchester, UK.

A novel approach combining homozygosity mapping with cartilage-specific gene expression analysis was used to identify the disease gene in a family with a unique form of extreme short stature. The 3 affected individuals showed a distinctive form of autosomal recessive spondyloepimetaphyseal dysplasia, characterized by severe short stature (22-24), flattened midface, prognathism, brachydactyly with joint laxity, and lumbar lordosis. Carriers had proportionate mild short stature. SNP array-based homozygosity mapping identified a 17Mb disease gene interval on chromosome 15, a region containing 296 genes. The genes were assessed and ranked by cartilage-selectivity using whole genome expression microarray data, revealing only 2 genes that were selectively expressed in cartilage: aggrecan (ACAN) and chondroitin sulfate proteoglycan 4 (CSPG4). Sequence analysis revealed homozygosity for a missense mutation (6793G>A) that predicts a D2265N amino acid substitution in the C-type lectin domain (CLD) within the G3 domain of ACAN. This residue is highly conserved among ACAN orthologues as well as paralogous lectican proteins. Expression of the normal and mutant G3 domains in mammalian cells showed that the mutation created a functional N-glycosylation site but did not adversely affect protein trafficking and secretion. The D2265 residue coordinates binding of a calcium ion which influences the conformational binding loops of the CLD, a domain known to mediate interactions with tenascins and other extracellular matrix proteins. Surface plasmon resonance studies showed that the mutant form of the ACAN G3 domain bound tenascin-C similar to the wild type protein, but dissociated slowly. These findings identify a new autosomal recessive skeletal dysplasia and a significant role for the aggrecan C-type lectin domain in regulating endochondral ossification.

Von Hippel-Lindau disease gene mutations in South African families : Identification of a founder mutation. *E. J. van Rensburg, C. M. Dorfling, M. de la Rey* Dept Human Genetics, Univ Pretoria, Pretoria, South Africa.

Von Hippel-Lindau (VHL) disease is a dominantly inherited familial cancer syndrome characterized by retinal and central nervous system haemangioblastomas, renal cell carcinomas, phaeochromocytomas, pancreatic endocrine tumours and endolymphatic sac tumours. In addition epididymal, pancreatic, and renal cysts are common. Since the *VHL* gene, located on chromosome 3p25, was cloned many inactivating mutations have been identified in VHL families. In two thirds of the families, missense, nonsense and splice site mutations as well as micro-deletions and micro-insertions have been identified. Large deletions involving part of or the entire gene, account for one third of the families. Thirteen South African families diagnosed on the basis of clinical criteria, were tested for *VHL* mutations in order to determine the frequency and spectrum of germ-line mutations. Following informed consent and extraction of DNA from peripheral blood, the coding exons and exon-intron boundaries were directly sequenced. Screening for large deletions was carried out using long-range PCR in which three overlapping fragments of 8.7Kb, 12.5Kb and 16Kb were amplified. Germ-line mutations were detected in all of the families; two missense (p.R167Q and p.C162F), one splice-site (c.463+1G>C), one novel in-frame insertion (c.591insGAT, p.197insD) and two novel large deletions of 5Kb and 6.3Kb, that both result in the in-frame deletion of exon 2, were detected. The breakpoints were sequenced and narrowed to homologous sequences of 7bp and 17bp respectively. With the exception of the 6.3Kb deletion that was detected in eight Afrikaner families, all other mutations were detected in one family each. Genealogical investigation of the eight families dating back to the 16th century have identified the founding couple for this mutation. To our knowledge, this is only the second founder mutation to be detected. A founder mutation (c.292C>T ; p.Y98H) has once before been described in families from Germany. These results now allow for presymptomatic testing of family members who can benefit from early diagnosis and treatment of VHL associated tumours.

Folinic acid-responsive seizures is identical to pyridoxine-dependent epilepsy. *R. Gallagher[1], J. Van Hove[1], G. Scharer[1], K. Hyland[2], B. Plecko[3,6], P. Waters[4], S. Mahmutoglu[3], S. Stockler-Ipsiroglu[3], G. Salomons[5], E. Rosenberg[5], E. Struys[5], C. Jakobs[5]* 1) Clinical Genetics and Metabolism, University of Colorado at Denver and Health Sciences Center, Denver, Colorado, USA; 2) Horizon Molecular Medicine, Atlanta, Georgia, USA; 3) Division of Biochemical Diseases, University of British Columbia, Vancouver, British Columbia, Canada; 4) Biochemical Genetics Laboratory, University of British Columbia, Vancouver, British Columbia, Canada; 5) Metabolic Unit, Department of Clinical Chemistry, VU University Medical Centre, Amsterdam, The Netherlands; 6) Department of Pediatrics, Medical University Graz, Austria.

Folinic acid-responsive seizures and pyridoxine-dependent epilepsy are two treatable causes of neonatal epileptic encephalopathy. The former is diagnosed by characteristic peaks on CSF monoamine metabolite analysis, its genetic basis has remained elusive. The latter is due to -aminoadipic semialdehyde dehydrogenase deficiency, due to mutations in the *ALDH7A1* (antiquitin) gene. We report two patients whose CSF showed the marker of folinic acid-responsive seizures, but who responded clinically to pyridoxine. We performed genetic and biochemical testing of samples from these individuals, and seven others, in order to determine the relationship between these two disorders. CSF was analyzed for -aminoadipic semialdehyde and pipecolic acid. DNA sequencing of the A*LDH7A1* gene was performed. Both patients had mutations in the *ALDH7A1* gene consistent with pyridoxine-dependent epilepsy due to -aminoadipic semialdehyde dehydrogenase deficiency. CSF samples from seven others, also diagnosed with folinic acid-responsive seizures because of characteristic CSF peaks, were identified to have elevated -aminoadipic semialdehyde, and sequencing of the *ALDH7A1* gene also revealed disease-causing mutations in these. These findings identify the cause of folinic acid-responsive seizures as -aminoadipic semialdehyde dehydrogenase deficiency due to mutations in the *ALDH7A1* gene, and demonstrate that folinic acid-responsive seizures is identical to the major form of pyridoxine-dependent epilepsy.

Automated Sequence Analysis Pipeline (ASAP) for Clinical Genetic Testing. *A. P. DeLuca[1], D. J. Wilder-Tack[1], N. E. Stone[1], K. R. Taylor[1], J. D. Bogaard[1], T. E. Scheetz[1], T. L. Casavant[1], V. C. Sheffield[1,2], E. M. Stone[1,2], T. A. Braun[1]*
1) University of Iowa, Iowa City, IA; 2) Howard Hughes Medical Institute, Chevy Chase, MD.

The Carver Non-profit Genetic Testing Laboratory (CNGTL) provides genetic testing to patients with inherited eye disorders. To minimize errors, genetic testing currently uses experienced personnel to manually read chromatograms to find and annotate mutations. As the volume of patients for genetic testing at the CNGTL has grown we have developed a system to augment the manual reading to improve workflow, laboratory information management, and add software-based mutation identification in addition to human sequence reading. This system detects mutations by leveraging existing bioinformatic software. The end result will be an increase in our capacity to perform genetic testing. Additionally patients will receive timely status updates on their tests and observed allelic variation frequencies will be made available to the community. Existing tools to detect variations in Sanger sequencing have either focused on large-scale variation discovery projects where the contents of a single chromatogram are not as important as finding a novel variant (PolyPhred, PolyScan), or tools that analyze datasets interactively (MutationSurveyor, InSNP). ASAP consists of a user interface that allows clients to view and transfer data, a database that acts as a central repository for the chromatograms as well as all associated annotation, and a series of servers that handle mutation detection (using PolyPhred), annotation (using BLAT and the UCSC annotation database), and integration with laboratory information management systems. It also improves the ability to evaluate sequence quality and identify sequencing problems more rapidly. ASAP is designed to analyze a continuous flow of chromatograms from genetic testing, and identify and annotate mutations. It generates mutations annotated in standard format defined by the Human Genome Variation Society (HGVS). In summary, ASAP enables clinical genetic testing to be performed more efficiently by reducing the burden of manual sequence reading.

A comparison of genome-wide imputation methods using the WTCCC control datasets. *B. Neale[1,2,3], Genetic Analysis Information Network(GAIN) Imputation Workgroup* 1) Richard Simches Bldg CPZN 6818, Ctr Human Gen Res, Massachusetts General Hospital, Boston, MA; 2) 7 Cambridge Center, Broad Institute of Harvard and MIT, Cambridge MA; 3) Institute of Psychiatry, Kings College London, De Crespigny Park, London, United Kingdom.

Imputation methodology has become pervasive in genome-wide association studies as a tool for checking data quality, increasing power, and facilitating meta-analysis by allowing cross-chip comparisons. However a rigorous comparison of the strengths and weaknesses of available methods using empirical data has not been performed. Generally, these methods utilize linkage disequilibrium patterns in the human genome to predict genotypes at untyped loci. Essential to the integrity of such efforts is the accuracy of the imputation algorithm. Given this wide array of applications, many groups have developed and implemented imputation methods. As part of the GAIN initiative, we have attempted to determine the relative merits of a majority of these methods. To do so, we are using the WTCCC 1958 birth cohort data, which has genotypes on the Affymetrix 5.0 and Illumina 550K platforms. Each of the ten enrolled groups run their respective imputation software on the Affymetrix 5.0 data to impute the Illumina 550K SNPs in HapMap. As outcome metrics, we use the accuracy of the posterior probabilities and allele frequencies estimated by the imputation methods. We also determine the number of individuals with posterior probabilities above thresholds for an assessment of the 'certainty' of each method. These three considerations are calculated for all SNPs and then broken down by minor allele frequency to shed light on the efficacy of imputation across the frequency range of human diversity. Additionally, we assess the relative computational requirements for each of these packages by running each method on a central computational system. In summation, we provide here insight into the general performance of imputation in different frequency and LD scenarios; the relative strengths of various methods in those scenarios; and introduce correction factors/recommendations for incorporating post-imputation uncertainty in meta-analysis.

Maternal germline mosaicism and somatic mosaicism in NF1. *M. R. Wallace[1], R. Loda[1], S. A. Rasmussen[1], K. Stephens[2], V. M. Riccardi[3]* 1) Molec Genetics & Microbiol, Univ Florida, Gainesville, FL; 2) Laboratory Medicine, Univ Washington, Seattle, WA; 3) The Neurofibromatosis Institute, La Crescenta, CA.

NF1 is an autosomal dominant condition present in 1/3000 - 1/4000 worldwide, characterized primarily by the presence of neurofibromas and café-au-lait spots, with an increased risk of learning disabilities and certain malignancies. Approximately half of newly-diagnosed cases of neurofibromatosis 1 (NF1) represent the first case in the family due to a new mutation in the NF1 gene. Although new-mutation cases are thought to have a constitutional NF1 mutation, an unknown percentage is actually mosaic for a somatic mutation that occurred early in development. There is a wide spectrum of NF1 mutations, with only one (microdeletion spanning NF1 and flanking genes) representing more than 1-2% of alleles. We present a unique family with an unaffected mother (without findings of NF1) with two affected children who are half-siblings. Both affected individuals have a novel Q2319X nonsense mutation in exon 38, but are highly discordant for NF1 features, particularly cutaneous neurofibroma burden. The mutation was not evident in the mothers blood by sequence analysis. Furthermore, a third child, an unaffected half-sib of one of the affected children, shares the same maternal allele, but lacks the Q2319X mutation, confirming that the mothers germline is truly mosaic with gametes representing 3 different NF1 alleles. We also describe somatic mosaicism in relatively mildly-affected new-mutation cases, including a novel frameshift in exon 29 (c.5382insG), and discuss the implications of mutation mechanism, effect on phenotype, and modifier loci.

Molecular Studies on FUNDC2 a candidate gene for mitochondrial diseases. *C. Laperuta, J. Monfregola, D. Esibizione, C. Carbone, M. V. Ursini, M. G. Miano* Institute of Genetics and Biophysics Adriano Buzzati Traverso CNR, Naples, Italy.

Human chromosome Xq28 is a gene-rich region, which lies within the critical linkage interval for many human diseases and characterized by an unusual degree of genomic instability. Here we report the structural/functional analysis of a new Xq28 gene FUNDC2 that encodes for a small mitochondrial protein with unknown function. The gene maps approximately 4,1 kb centromeric to F8 and 6,5 kb telomeric to MTCP1. Comparative and chromosomal mapping indicates that FUNDC2 has duplicated to specific locations near the pericentromeric region of human chromosomes highlighting a phenomenon for paralogous genome evolution common to other Xq28 genes. FUNDC2 has ubiquitously expression in human embryos and adult tissues with high level in the heart and skeletal muscle. Its predicted protein shows high conservation from Archeabacteria to Human suggesting that it could be a member of a new family of functionally related genes. Its 189-amino-acid sequence appears rich of hydrophobic residues and lacks a signal-sequence for protein sorting or target membrane. In HeLa and COS cells, confocal immuno-fluorescence studies co-localized FUNDC2 protein to the mitochondria stained by anti-HSP60 antibody and pECFP-mito. We also performed subcellular fractionation by differential centrifugation and alkali treatment establishing that FUNDC2 is located in the mitochondria inner membrane. Based on a high-throughput yeast 2-hybrid screen, we found that Drosophila FUNDC2 orthologue interacts with mtacp1 (mitochondrial acyl carrier protein 1). Its human counterpart is NDUFAB1, a subunit of the mitochondrial respiratory Complex I that is the entry point for electrons donated from NADH. Mutations in the structural building blocks of Complex I have been detected in 40 percentage of patients with Complex I deficiencies. Further investigation of FUNDC2 will contribute to understanding of its function as well as provide a new candidate for mitochondrial pathogenesis including those associated to Complex I deficiency.

Mitochondrial DNA footprints in modern Mongolia. *S. R. Woodward[1], A. Achilli[2,3], U. A. Perego[1,3], J. E. Gomez-Palmieri[1], D. Tumen[4], E. Myagmar[4], D. Bayarlhagva[4], K. H. Ritchie[1], A. Pollock[1], N. Angerhofer[1], A. Torroni[3], N. M. Myres[1], Sorenson Molecular Genealogy Foundation, SLC, UT (USA)* 1) Sorenson Molecular Genealogy Foundation, Salt Lake City, UT, USA; 2) Dip. di Biologia Cellulare e Ambientale, Università di Perugia, Perugia, Italy; 3) Dip. di Genetica e Microbiologia, Università di Pavia, Pavia, Italy; 4) National University of Mongolia, Ulan Bator, Mongolia.

Although Mongolia is one of the most sparsely populated countries in the world, it is located at a pivotal crossroad between the four corners of Asia (including the well-known Silk Road) and has been characterized throughout history by events that greatly added to its current cultural and ethnic diversity. Among these, perhaps one of the most significant happening was the ambitious expansion strategy employed by Mongolias most prominent personality, Genghis Khan, whose empire eventually stretched across all of modern-day China, a portion of modern Russia, Southern Asia, Eastern Europe and the Middle East. In 2007, through a well-planned collection effort, researchers at the Sorenson Molecular Genealogy Foundation and the National University of Mongolia were able to gather over 3,000 DNA samples, informed consents, and genealogical data throughout the country of Mongolia, including samples from 21 distinct tribal or ethnic populations. All the samples were sequenced for the three hypervariable segments of the mitochondrial DNA (mtDNA) control region to assess the genetic composition of modern Mongolia. The most common mtDNA haplotypes are typical of haplogroup C, which is frequent throughout Eastern Asia. However, nearly 40% of the observed mtDNA lineages are of Western Eurasian origin, including a significant frequency (~7%) of haplogroup H - the most common in Europe. The high prevalence of Western Eurasian lineages could be a remnant from Genghis Khans conquering efforts, trade and cultural exchanges along the Silk Route. To assess the extent of recent gene flow that could account for the elevated levels of Eurasian haplogroups within Mongolian populations, we have examined genealogical data of samples representative of Western Eurasian haplogroups.

Incontinentia pigmenti caused by NAHR in NEMO locus: a case report. *F. Fusco[1], A. Pescatore[1], M. Paciolla[1,2], MB. Lioi[2], MG. Miano[1], MV. Ursini[1]* 1) Institute of Genetics and Biophysics ;Adriano Buzzati-Traverso; (IGB-CNR), Naples, Italy; 2) University of Basilicata, Potenza, Italy.

Incontinentia Pigmenti (IP) is an X-linked dominant disease caused by mutation in the NEMO gene located in the Xq28 chromosomal region. NEMO encodes for a key subunit of the crucial IKK regulatory complex required for the activation of NF-kB pathway. Therefore, the remarkably heterogeneous and often severe clinical presentation reported for IP is due to the pleiotropic role of this transcriptional signalling pathway. Previous reports from us and from others have demonstrated that 70 percentage of the IP cases are due to a recurrent exon 4-10 genomic rearrangement in the NEMO gene. We report on a case of Incontinentia Pigmenti in an 8 months old girl who presented the classical dermatological four signs of the disease associated to neonatal seizures. The analysis of the NEMO gene responsible for IP in the patient DNA revealed the presence of a common exon4-10 deletion which was not present in the unaffected mother of the girl who was instead carrier of an exon4-10 deletion in the closely located non-functional NEMO pseudogene. Haplotype analysis of the NEMO/pseudoNEMO region in the mother of the proband, the grandmother and other unaffected members of the family revealed that a homologous non-allelic recombination between the NEMO gene and pseudogene mediated the relocation of the exon4-10 deletion from the pseudogene to the gene. This is the first case of a genomic IP rearrangement caused by nonallelic homologous recombination (NAHR) between gene-pseudogene pair in the NEMO locus and enrols IP among the very few disease caused by gene conversion between gene-pseudogene pair. It also represents a significant contribution to highlight how unaffected individuals carrying deletions in the pseudoNEMO may be at risk to have descendants with IP.

Multi-ethnic Genome-wide Alterations in Breast Cancer using Paraffin Embedded Samples. *M. E. Ahearn[1], C. Gomez[1], M. Jorda[1], T. Halsey[2], J. Yan[2], A. Mejias[1], K. Ellison[2], K. Mulligan[2], M. Pegram[1], S. Gluck[1], L. Baumbach[1]* 1) Univ Miami, Miami, FL; 2) Almac Diagnostics, Durham, NC.

Ethnic-specific disparities in breast cancer (BC) stage of presentation and survival rates are well documented. To investigate possible ethnic-specific genetic contributions to disparity, we are completing gene expression profiling of a multi-ethnic cohort consisting of 30 Triple Negative BC patients [10 each African-American (AA), Hispanic (His) and non-Hispanic white (Cauc) women] matched for age of diagnosis. The overall aim is to increase understanding of the biological basis of ethnic-specific BC disparities, leading ultimately to ethnic-specific diagnostic and therapeutic approaches. Immediate goals are to demonstrate utility of FFPE samples in obtaining consistent, reproducible data from gene expression arrays, and to identify differentially expressed genes between tumor and normal tissue common or unique to the ethnic groups. Specimens from FFPE blocks were marked by pathology as to normal vs. tumor tissue. RNA isolation, cDNA preparation, and hybridization of tumor/normal cDNAs to a breast cancer focused gene expression microarray (*Breast Cancer DSA Research Tool*) was performed by *Almac Diagnostics*. Using self matched tumor and normal FFPE samples from 18 patients, over 17516 transcripts were detected on the *Breast Cancer DSA* with intensity significantly greater than background. For normal and tumor tissue, 9399 and 10,296 transcripts respectively, were detected in all three groups. Importantly, a subset of transcripts was detected in only one or two ethnic groups allowing identification of ethnic-specific expression patterns in the matched normal/tumor tissue samples. We are completing this study by increasing sample size, matching for stage, mapping clusters of differentially-expressed genes in pathway analysis, and validation by real-time PCR. DNA copy number variation (CNV) will be investigated by high density SNP arrays. This preliminary analysis shows that: high quality gene expression data can be generated from FFPE samples, and ethnic specific gene expression differences can be detected in tumor and matched normal breast tissue samples across ethnic groups.

Questions raised by molecular genetic testing for Huntington disease: 10 years-experience of a reference diagnostic laboratory in Portugal. *I. Alonso[1,2], J. Cerqueira[2], E. M. Ramos[1], P. Magalhães[2], M. C. Costa[1], J. Pinto-basto[1,2], J. Sequeiros[1,2,3]* 1) Unigene, IBMC - Univ Porto, Porto, Portugal; 2) CGPP, IBMC, Univ. Porto, Portugal; 3) ICBAS, Univ. Porto, Portugal.

Huntington disease (HD) is a degenerative neurological disease, with autosomal dominant transmission, with onset usually in adulthood. It is clinically characterized by involuntary choreic movements, motor and cognitive impairment and behavioural changes. Its molecular defect is a $(CAG)_n$ expansion in the coding region of the HD gene, on 4p16.3. CGPP is the reference laboratory for routine testing of HD in Portugal, since 1998. We have performed 1,084 molecular tests for HD, over the last 10 years: 737 were requests for confirmation or exclusion of a clinical diagnosis; 320 were presymptomatic, and 16 were prenatal diagnostic tests. Proficiency testing has been evaluated every year, through the external quality assessment schemes of the European Molecular Genetics Network (www.emqn.org). Only 58.4% of all diagnostic requests were confirmed, as many came just for exclusion, in the differential diagnosis of HD. Among all presymptomatic tests, 40.3% of the subjects were found to be carriers; an excess of female consultands (65.3%) was observed. In the 16 prenatal tests performed, seven foetuses carried the mutation (one couple abandoned the counselling process, just before delivery of the test result). We found a full homozygote (with 41 and 51 CAGs), as well as three cases compound by one reduced-penetrance and one fully-mutated allele. Alleles of reduced penetrance (36-39 CAGs) were found in 24 cases. Normal homoallelism (two wild-type alleles of the same size) was seen in approximately 6% of all cases, and represents a major concern particularly in presymptomatic and prenatal testing. It requires the use of additional testing and techniques for the exclusion of a large expansion, which are continuously under development. Other important concerns, regarding genetic counselling, are the finding of large normal alleles, reduced-penetrance alleles, or a compound of these or of these with full expansions, as well as the presence of two expansions, particularly in patients already with offspring.

Molecular basis of Y-linked hearing impairment (DFNY1) in a Chinese pedigree. *C. Tyler-Smith[1], Y. Xue[1], -. Asan[1], Q. Long[1], D. J. Turner[1], F. Yang[1], M. Quail[1], B. L. Ng[1], N. P. Carter[1], H. Yang[2], Q. Wang[3]* 1) The Wellcome Trust Sanger Institute, Hinxton, UK; 2) Beijing Genomics Institute, Beijing, China; 3) Department of Otolaryngology, Head and Neck Surgery, Chinese PLA Institute of Otolaryngology, Beijing, 100853, China.

Y-linked hearing impairment has previously been described in a large Chinese family (Wang et al. (2004) J. Med. Genet. 41 e80). We have now investigated the molecular basis of this phenotype. We discovered an unaffected branch of the pedigree and used 67 Y-STRs to verify that it carried the same Y-chromosomal lineage, then set out to find mutational differences between affected and unaffected Y chromosomes. The two Y chromosomes were flow-sorted and completely resequenced using Solexa/Illumina paired-end reads. No sequence differences in protein-coding genes were found, but read-depth measurement indicated duplication of two discontinuous regions (adding up to about 0.5 Mb) in proximal Yp. Duplication was confirmed by high-resolution oligonucleotide array CGH, quantitative PCR, pulsed-field gel electrophoresis and fibre-FISH. The duplication has not been reported in individuals with normal hearing and maps to the same position on the pedigree as the hearing-impairment phenotype, so is likely to be causal. The only annotated protein-coding genes included in the duplicated region are part of the TSPY1 gene cluster.

Epigenetic changes in Friedreich ataxia are associated with altered CTCF binding in the FXN gene promoter. *I. De Biase, P. Rindler, Y. Chutake, S. I. Bidichandani* Biochemistry & Molecular Biology, University of Oklahoma Health Sciences Center, Oklahoma City, OK.

Friedreich ataxia (FRDA) patients are homozygous for large expansions of a GAA triplet-repeat (GAA-TR) sequence in the FXN gene. The expanded alleles severely reduce FXN gene transcription, resulting in deficiency of the mitochondrial protein frataxin. The repeat induces heterochromatin formation in vivo. Hallmarks of silenced genes have been described in FRDA patient cells and transgenic mouse models, including increased DNA methylation and histone hypoacetylation and trimethylation. Pharmacological reversal of heterochromatin changes increases frataxin mRNA and protein levels. We show that the FXN gene promoter is a target for epigenetic changes that may allow the spreading of heterochromatin within this region. We identified a binding site for CCCTC-Binding Factor (CTCF), an established vertebrate genomic insulator, in the minimal promoter region of the human FXN gene, which is also conserved in P. troglodytes. CTCF acts as transcriptional insulator, organizing eukaryotic genes into domains. We confirmed the binding of CTCF to the frataxin promoter in vitro and in vivo using electrophoretic gel mobility shift assay, and chromatin immunoprecipitation (ChIP) followed by real-time PCR, respectively. Furthermore, CTCF binding to the FXN gene promoter was significantly reduced in two FRDA fibroblast cell lines versus two healthy control cell lines. There was no difference in the promoter methylation status between patients and controls, and in vitro methylation of the binding site did not alter CTCF binding, excluding this mechanism as responsible for the reduced CTCF binding. Furthermore, using ChIP analysis we found an increase in the trimethylation of histone H3 at lysine 9 and 27 specifically in the FXN gene promoter of FRDA patients, which strongly correlates with silenced chromatin. Our results indicate that the GAA expansion in FRDA alters CTCF binding in the FXN gene promoter, and a potential role for CTCF in regulating FXN transcription and local chromatin organization.

The ITMAT-Broad-CARE (IBC) array: a 50K candidate gene SNP array for large-scale CVD association studies. *B. J. Keating[1], S. Tischfield[2], T. Bhangale[3], S. S. Murray[4], T. S. Price[1], J. Barrett[5], M. I. McCarthy[5], M. R. Reilly[1], J. C. Engert[6], D. J. Rader[1], D. A. Nickerson[3], J. N. Hirschhorn[2], G. A. FitzGerald[1]* 1) Inst Translational Med/Therap, Univ Pennsylvania, Philadelphia, PA; 2) Broad Institute of Harvard & MIT, Cambridge, MA 02142, USA; 3) Dept of Genome Sciences, Uni. of Washington, Seattle, WA 98195, USA; 4) Scripps Genomic Medicine, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA; 5) The Wellcome Trust Centre for Human Genetics, Uni. of Oxford, OX3 7BN, UK; 6) Dept of Medicine & Human Genetics, McGill Uni., Montréal, Québec, Canada.

A wealth of associations for CVD phenotypes has been accumulating, in particular a large number of loci derived from recent GWAS.True complex disease-associated loci often exert modest effects, so their delineation requires integration of diverse phenotypic data from large studies to ensure robust meta-analyses.We have designed a ~50K SNP array (Infinium, Illumina) to assess potentially relevant loci across a range of CVD syndromes.The array utilizes a cosmopolitan tagging approach to capture genetic diversity in ~2,100 loci in populations represented in HapMap & SeattleSNPs. The array content is informed by vascular & inflammatory disease GWAS, CVD implicated eQTLs & comprehensive literature searching.The custom flexibility of the array platform facilitated interrogation of loci at differing stringencies allowing saturation of higher priority loci with denser markers than existing GWAS tools.We demonstrate that the array can be used effectively in complementation with GWAS to increase coverage in high priority loci.Recent efforts show that CNVs can be accurately mapped on the platform using signal intensity metrics.Over 1500 loci are tagged at denser coverage than most commercial GWAS tools & we demonstrate multiple novel CNVs in these loci at high resolution using CNV calling algorithms including PennCNV. DNA from >200K extensively phenotyped individuals are being genotyped on the array (80K at time of press) with significant portions of data being released into the academic domain in 2008 facilitating in silico replication, analyses of rare variants, meta-analyses in diverse populations & epistasis.

Newborn Screening for Fragile X Syndrome and Sex Chromosome Aneuploidies by FMR1 DNA Methylation. *B. Coffee, T. Malone, K. Keith, J. Mowrey, S. T. Warren* Department of Human Genetics, Emory University, Atlanta, GA.

The vast majority of fragile X syndrome is due to aberrant changes in chromatin structure, as a consequence of CGG repeat expansion, resulting in the silencing of the FMR1 gene. One epigenetic change concomitant with CGG repeat expansion is DNA methylation of FMR1. We have developed a rapid, high-throughput and quantitative methylation-sensitive PCR method (Q-MSP) to assess FMR1 methylation in DNA from dried blood spots from newborn boys to screen for fragile X syndrome. An additional benefit of this method is that it will also detect numerical and structural sex chromosome aneuploidies, such as Klinefelter syndrome (47,XXY). The sensitivity of the Q-MSP method allows for the pooling of male samples, permitting the simultaneous screening of 44 males in a single experiment. Given the incidence of these abnormalities, the majority of these pools will screen negative requiring no further analysis, reducing the cost of screening substantially. Positive pools are then rescreened to identify the positive individual who is then verified, using PCR and Southern analyses on fresh DNA extracted from the original dried blood spot. An advantage of this approach is that Q-MSP directly detects abnormal FMR1 DNA and circumvents the problems with long CGG-repeat amplification. In addition, this approach specifically will not detect newborn males who are premutation carriers, and potentially at risk for fragile X associated tremor ataxia syndrome, as we consider it inappropriate to identify newborns at risk for adult neurodegenerative disease. To test the feasibility of his methodology for population screening, we have collected and are screening a cohort, currently consisting of 40,000 deidentified male blood spots, collected from the Georgia Public Health Laboratory. Using this approach we have identified positive individuals who were verified by secondary analysis to have fragile X syndrome or sex chromosome abnormalities. Preliminary prevalence estimates will be presented using the latest completed dataset.

Determination of Airway Epithelium Expression Levels of Glutathione S-transferase Subtype M by Copy Number Variation Polymorphisms. *N. Hackett, T. O'Connor, J. Salit, Y. Strulovici-Barel, R. Crystal* Dept Gen Med, Weill Cornell Med Col, New York, NY.

Smoking is the major risk factor for chronic obstructive pulmonary disease (COPD), but only 15-20% of chronic smokers develop the disease. Given that the initial manifestations of COPD occur in the small airway, we hypothesize that the gene expression patterns for protective and susceptibility genes in the small airway epithelium of smokers determines risk for COPD. Further, we propose that the gene expression level for these genes is genetically determined by polymorphisms including copy number variation in the vicinity of the gene. Glutathione is proposed to play a critical role in protection of the airway epithelium from oxidant damage and glutathione S-transferase polymorphisms have been reported to increase risk for COPD (J Zidzik et al, Croat. Med J 2008; 49:182). Genomic copy numbers in the vicinity of the glutathione S-transferase M gene cluster in chromosome 1 (nucleotides 110,000 - 110,085 kb) were determined using the Affymetrix Genome-Wide Human SNP Array 5.0. Gene expression levels were determined by analysis of airway epithelium mRNA levels on the Affymetrix Human Genome U133 Plus 2.0 microarray. Of our population of 86 smokers, 21% were observed to have deletions of both alleles encompassing approximately 30 kilobasepairs within the GSTM gene cluster. Expression levels for GSTM1 and GSTM3 in these subjects were significantly lower ($p<0.01$ both genes) than in subjects with at least 1 copy of the gene. By contrast, the expression level for flanking genes (GNAI3 and AHCYL1) were the same ($p>0.5$) for subjects with and without the GSTM deletions. Therefore, high frequency copy number variation polymorphism can dictate the expression levels of genes critical to the protection of the airway from smoking-induced oxidant damage. This suggests the link between GSTM polymorphisms and COPD is mediated by airway epithelial GSTM expression levels.

A novel 1p32 microdeletion in patients with abnormalities of the corpus callosum. *C. Haldeman-Englert[1], D. M. McDonald-McGinn[1], J. Coppinger[2], L. G. Shaffer[2], S. Kubendran[3], S. G. Kahler[3], P. L. Brock[4], G. C. Gowans[4], R. P. Matthews[5], E. H. Zackai[1], T. H. Shaikh[1]* 1) Human Genetics, Children's Hosp of Philadelphia, Philadelphia, PA; 2) Signature Genomics Laboratories, LLC, Spokane, WA; 3) Pediatrics, Univ of Arkansas for Medical Sciences, Little Rock, AR; 4) Univ of Louisville, Louisville, KY; 5) Gastroenterology, Children's Hosp of Philadelphia, Philadelphia, PA.

The introduction of microarray-based DNA analysis has greatly accelerated the discovery of many novel genomic syndromes. These rearrangements frequently lead to congenital anomalies and/or mental retardation by presumably altering the dosage of one or more genes. Using high-density oligonucleotide microarrays, we have identified overlapping *de novo* deletions of 1p32.2-p31.3 in three patients, each with micrognathia and a corpus callosum (CC) abnormality. Patients 1 and 3 have an absent CC, a cleft palate, and a structural cardiac defect. Patient 2 has thinning of the posterior CC, and also has a 15q11.2-q13.1 microdeletion of the Prader-Willi/Angelman syndrome region. The smallest overlapping deleted region is 371 kb and includes only one known transcript, *FGGY*. The function of this gene is unknown, but protein homology suggests that it may be involved in carbohydrate metabolism. To elucidate the function of this gene in normal development, we used the zebrafish as an animal model. Preliminary results using two morpholinos against *FGGY* show a similar abnormal phenotype, and studies are ongoing to further characterize these morphans. Another gene, *NFIA*, is also deleted in Patients 1 and 2. Haploinsufficiency of this gene has been previously reported in two patients with similar deletions and CNS abnormalities. After review of the literature, a total of 8 patients (including 3 patients from the present study) have been identified with deletions of this region. They appear to have variable clinical phenotypes but share similar CNS malformations with the CC commonly involved. Additional investigation of the genes in this region may provide further insight into these patients phenotypes as well as their role in early brain development.

Limb and craniofacial abnormalities and a patient with chromosome 1q21.2-21.3 deletion. *H. Vernon[1, 2], K. Johnson[2], V. Kottoor[1], E. Lisi[1], G. Cutting[1], D. A. S. Batista[3,4]* 1) Institute of Genetic Medicine; 2) Department of Pediatrics; 3) Department of Pathology, Johns Hopkins Hospital; 4) Kennedy Krieger Institue, Baltimore, MD.

We report a patient with multiple congenital anomalies and an interstitial deletion of chromosome 1q. The patient was born to a nonconsanguineous couple after an uneventful full term pregnancy. She was noted to have multiple dysmorphic features at birth. After further clinical investigation she was found to have multisystem organ dysfunction including: congenital heart defect (ventricular septal defect), craniofacial abnormalities (severe micrognathia and retrognathia, malar hypoplasia), renal abnormalities (multicystic dysplastic kidney), hematologic abnormalities (anemia) and skeletal abnormalities (bilateral small radii and ulnae, fused radius and ulna, unilateral absent thumb on left, fused on right, unilateral missing digit). A karyotype was performed and initially described as normal, 46, XX. A 4,200 BAC array-CGH was performed because an underlying genetic defect was strongly suspected. The patient was found to have a deletion of chromosome 1at bands q21.2-21.3. The size of the deletion was estimated to be between 2.3 and 5.6 Mb. After this deletion was found, chromosome 1 was re-examined in the karyotype, and in some elongated chromosomes a deletion could be suspected. Parental chromosome analysis is pending. A patient with similar clinical features and deletion of chromosome 1q12-1q21.3 was described previously (Waggoner et. al, 1999, Am J Med Genet 82:301-4). This patient had radial ray abnormalities, similar craniofacial abnormalities, and a cardiac defect. This region is also of particular interest because the suspected critical region for susceptibility for TAR (thrombocytopenia absent radius) syndrome is located on 1q21.1, which either overlaps or is immediately proximal to the centromeric area of the common region of these patients deletions. The discovery of a discrete deletion in our patient provides new evidence that the 1q21 region harbors genes responsible for radial limb development.

ALFRED: a resource for research and teaching. *K. K. Kidd[1], H. Rajeevan[1], K.-H. Cheung[2], U. Soundararajan[1], S. Stein[1], A. J. Pakstis[1], J. R. Kidd[1]* 1) Dept Genetics, Sch Med, Yale U, New Haven, CT; 2) Ctr Med Informatics, Sch Med, Yale U, New Haven, CT.

ALFRED (http://alfred.med.yale.edu) is a unique resource for research and teaching in human genetics and related areas. ALFRED is free, web-accessible, actively curated and GIS-enabled with links to ethnographic and molecular databases, and to the relevant literature. As of June 2008 ALFRED has data on 14,617 polymorphisms and 653 different populations(with multiple samples for 359 populations), a total of 277,127 frequency tables(one population sample typed for one site). 22 markers in ALFRED have frequency data on over 100 population samples; the 32bp Ins/Del in coding region of gene CCR5 has data on 226 different population samples. In the past year data from high throughput datasets have been added to ALFRED increasing the type and quantity of data considerably. This unique collection of allele frequencies has been integrated with other genetic databases by reciprocal URL links to and from PharmGKB, dbSNP and Genopedia HuGE Navigator. ALFRED description pages can be accessed from these resources. Mapping tables for gene identifiers (Entrez Gene ID, ALFRED UID) and polymorphism identifiers (dbSNP rsnumber, ALFRED UID) have been provided for ease of linking to ALFRED pages from other resources. This mapping information offers an immensely useful tool for researchers and bio-informaticists to map SNP/gene information obtained from NCBI to frequency data downloaded from ALFRED. Because earlier literature may have a SNP associated with a gene due to proximity but now is known to be in an adjacent gene, a polymorphism can be associated with a gene in many ways: as the primary locus, as also associated with, or as previously associated with. Fst and avg heterozygosity values for bi-allelic SNPs are pre-calculated and displayed in tables organized by chromosome. Individual locus values can also be viewed from each Polymorphism Description page. In addition to providing genotype summary data ALFRED now has a link to individual genotype data for several hundred SNPs on ~2000 individuals in 43 populations typed at Kidd Laboratory, Yale U. Support: US NSF BCS-0725180.

Folic acid supplementation during pregnancy in MTHFR c.1298CC homozygote mothers associates with an increased the risk of cleft lip and palate in the offspring. *M. Rubini[1], C. Baluardo[1], M. Ferrian[1], E. Calzolari[1], A. Franchella[3], M. Accordi[4], G. Garattini[2], R. Brusati[2]* 1) Eperimental and Diagnostic Medicine Dept. Medical Genetics Unit, University of Ferrara, Ferrara, Italy; 2) Maxillo-facial Surgery Dept., University of Milan, Milan, Italy; 3) Surgical Pediatrics Unit, Sant'Anna Hosp., Ferrara, Italy; 4) Phoniatric Center, Padova, Italy.

Folate metabolism plays a critical role in embryonic development, and folic acid (FA) supplementation during early pregnancy has been reported to reduce the risk for isolated cleft lip with/without cleft palate (CL/P) by a third. We used a case-parent triad design to determine if common variants in genes encoding folate enzymes were risk factors for CL/P and explore if folate supplementation during pregnancy interacts with mothers or childs genotype to modify the risk for CL/P. We analyzed 163 complete CL/P triads collected in Italy in the 1999-2002 period. All individuals were genotyped for MTHFR c.677C>T and c.1298A>C, MTR c.2756A>G, and MTRR c.66A>G variants. Association was assessed using transmission disequilibrium test (TDT) and relative risks of variant alleles in mother or child were calculated by log-linear Poisson regression model. Periconceptional supplementation with FA (alone or in multivitamins) was observed in only 8.6% of mothers, while in 27.6% cases FA administration begun after pregnancy ascertainment. None of the studied polymorphisms significantly associated with CL/P risk, but in MTHFR c.1298CC homozygote mothers the relative risk for CL/P was significantly increased ($p<0.0001$) when FA was administered at time of pregnancy ascertainment, while FA had no significant effect when supplementation begun before conception. In c.1298CC mothers FA administration during pregnancy was associated with a eight-fold increased relative risk for CL/P (RR = 7.98, 95%C.I. 3.45-18.4). Although FA supplementation in general reduces the risk of CL/P, this evidence suggests that in subgroup of cases beginning FA administration after pregnancy ascertainment might have opposite effect, and suggest that MTHFR c.1298A>C genotyping of mothers could be considered before administration of FA during pregnancy.

Prevalance of Slow Metablizing Cytochrome P450 CYP2C9*2 Allele in the Qatari Population may Impact Dosage of Warfarin. *T. O'Connor[1], N. Pereira[2], L. Chouchane[2], H. Sattar[3], M. Allangawi[3], A. Al-Mohammed[3], W. Ibrahim[3], T. Raza[3], M. Hamza[3], A. Al-Mulla[3], M. Al-Merri[3], A. Al-Hashemi[3], M. Al-Nesf[3], J. Geraghty[2], R. Mathew[2], A. Maayah[2], A. Gohar[2], N. Hackett[1], R. Crystal[1]* 1) Department of Genetic Medicine, Weill Cornell Med Col, New York, NY; 2) Weill Cornell Medical College-Qatar, Doha, Qatar; 3) Hamad Medical Corporation, Doha, Qatar.

Warfarin is a commonly prescribed anticoagulant drug that is used as a treatment for deep vein thrombosis and pulmonary embolism, and is also used prophylactically for the prevention of thromboembolic events that could arise following surgical procedures. Despite warfarins low cost and proven efficacy, there is considerable morbidity from warfarin therapy because of wide variation in the rates of metabolism of the drug. Based on the knowledge that the CYP2C9*2 and *3 alleles are poor metabolizers of warfarin (necessitating 15-30% lower dosage), and the knowledge that there are ancestral differences in prevalence (<1% Asians, 1-3% African-Americans, and 8-12% Europeans) of the CYP2C9*2 and *3 alleles, we asked: what is the prevalence of these alleles in the Qatari population, a population with a high rate of consanguinity that evolved from Arab, Persian and African populations? DNA extracted from blood samples of n=42 self-reported Qataris was assessed by TaqMan allelic discrimination assays (Applied Biosystems) for the CYP2C9*2 and CYP2C9*3 alleles. The analysis revealed a high prevalence of the CYP2C9*2 allele (frequency 0.16), compared to the prevalence of that allele in HapMap populations (frequency of 0.10 in European and absent in Chinese, Japanese and African). For the CYPC9*3 allele, the prevalence in the Qataris was 0.05, which is within the range of prevalence frequency in the HapMap populations (frequency of 0.04, 0.06, 0.03 and 0.00 in European, Chinese, Japanese, and African respectively). In neither case did the population genotype frequencies differ significantly from Hardy-Weinberg equilibrium ($p>0.2$). The Qatari population genotyped has a relatively high frequency of the CYP2C9*2 allele, an observation with clinical relevance to the use of warfarin in thromboses and in prophylactic applications in this population.

Method to Measure Global DNA Methylation. *D. Vassar* R&D, Genomics, Sigma-Aldrich Research Biotech, St Louis, MO.

It has been well demonstrated that DNA methylation plays an important role in the regulation of gene expression. Shifts away from normal global DNA methylation levels have been observed in various cancers, neurological disorders, autoimmune diseases, and aging. Several methods exist to measure global methylation levels but each have drawbacks including the cost of required equipment [i.e. mass spec methods], lengthy protocols [i.e. enzymatic degradation and analysis] and/or radioactive components. We have developed a new method, similar to a sandwich ELISA, which allows fast measurement of overall DNA methylation status. We utilize two models separately and in combination to demonstrate the utility of this new technique for global DNA methylation measurement. The first example employs representatives of the MicroRNA-29 family, which have been shown to decrease global DNA methylation in lung cancer cell lines. In addition, we have used decitabine, a well-known demethylating agent, to measure its effects on total DNA methylation in A549 cells. Both systems afford the expected levels of demethylation, and show the utility of this new, four-hour global methylation measurement method.

Association of CD14 Promoter Polymorphism with prostate cancer in men of African descent. *T. E. Mason[1], L. Ricks-Santi[1,6], V. Apprey[1], W. Chen[1], J. Joykutty[1], C. Ahaghotu[1,2], R. A. Kittles[3], G. Bonney[1,4], G. M. Dunston[1,5]* 1) National Human Genome Center at Howard University, Washington, DC; 2) Division of Urology, Howard University Hospital, Washington, DC; 3) Department of Medicine, University of Chicago, Chicago, IL; 4) Department of Community Health and Family Medicine, Howard University, Washington, DC; 5) Department of Microbiology, Howard University College of Medicine, Washington, DC; 6) Howard University Cancer Center, Washington, DC.

African American men have the highest rates of prostate cancer worldwide and immunogenetic studies suggest that people of African descent have increased susceptibility to diseases of inflammation. We hypothesize that sequence variants in the promoter region of the CD14 gene that regulate inflammation may modify individual susceptibility to prostate cancer. The CD14 promoter region was screened for SNPs using dHPLC. One variant, -260 C>T (rs2569190), was genotyped via restriction digest in all study participants (264 cases and 188 controls). Eleven variants (4 novel) were identified in the promoter region of CD14. A marginal association between the C allele and prostate cancer was found ($p=0.07$). When stratified by age, among men 55 years of age, the C allele was significantly associated with prostate cancer ($p=0.03$). When stratified by self-reported ethnicity, African-American men with the C allele were at a higher risk for Prostate Cancer ($p<0.05$) compared to those of African ancestry (Afro-Caribbean, Afro-Latino, East and West Africans). Here is the first study to show an association between the C allele in CD14 -260 variant and prostate cancer and supports the hypothesis that genetic variation in the inflammatory process can contribute to prostate cancer susceptibility. Our findings have interesting implications for the evolution of PRRs and regulation of the inflammatory response in health disparities. With the advent of genome-wide variation resources such as Hap Map offers unprecedented opportunities to investigate evolutionary forces that have shaped inherited variation in natural populations.

Microsatellite instability is not present in endometrial carcinoma cell lines after 2 and 4 Gy gamma irradiation.

M. Alves[1,7], J. Fialho[1,2], F. Ladeirinha[3,7], T. S. Silva[4,7], F. Botelho[5,7], C. Oliveira[6,7], I. M. Carreira[3,7], C. Monteiro[1,7]
1) Fac Farmácia, UL, Lisboa, Portugal; 2) IBEB, FCUL; 3) Fac Medicina, Univ Coimbra; 4) Serv Anat Patol, Hosp Univ Coimbra; 5) IBILI, Fac Medicina, Univ Coimbra; 6) Serv Ginecol, Hosp Univ Coimbra; 7) CIMAGO, Coimbra, Portugal.

Endometrial cancer (EC) is the commonest gynecological cancer in Europe and North America. Radiotherapy is the most effective conservative treatment for EC in women with high risk of surgery, though its efficiency is limited, as tumors exhibit different behavior and the appearance of resistant subpopulations upon relapse of an originally responsive malignancy can occur. In previous studies with lymphoblastoid cell lines [LB705 (normal), WI-L2-NS (TP53-/-) and LB463(XPG)] treated with ionizing radiation (), our group observed novel length alleles (microssatelite instability - MSI) in a TP53 *locus* marker and a decrease in the principal alleles height in the normal cell line. As these results suggest the emergence of radiation resistant neoplastic MSI+ mutant clones, we decided to investigate whether or not this phenomenon could be involved in the resistance to ionizing radiation in EC. So, matched tumoral and non-tumoral endometrial cells from 4 patients submitted to hysterectomy were established in culture. These cell lines were irradiated with the technetium isotope ^{99m}Tc at an effective dose of 2Gy and 4Gy. At days 0, 4, 8 and 12 post-irradiation the viability of the cells was determined with trypan blue and their MSI profile determined by amplification and fragment analysis of 5 mononucleotide repeats (NR-21, NR-24, NR-27, BAT-25 and BAT-26). A parallel control group of non-irradiated cell lines was also studied. The 4 tumors studied were all MSI- and no MSI was detected at any time post-irradiation, which contrasts with the results we found in XPG cell lines. Nevertheless, more cell lines are being irradiated in order to have a more representative picture of this carcinoma and to elucidate the mechanisms of its resistance to radiation. The generated results may allow the prediction of tumor responsiveness to radiotherapy and the adjustment of therapeutic protocols. M Alves is a post-doctoral fellowship from FCT.

Identification of GATA3 Regulated Transcription Factors in the Inner Ear. *D. Alvarado, R. Veile, M. Warchol, M. Lovett* Washington Univ, St Louis, MO.

The inner ear utilizes sensory hair cells as mechano-electric transducers for sensing sound and balance. In mammals, these hair cells lack the capacity for regeneration. However, hair cells from non-mammalian vertebrates, such as birds, can be regenerated throughout the life of the organism. Mutations in the zinc finger transcription factor GATA3 are known to cause sensory neural deafness in humans and we previously identified GATA3 expression differences in the cochlea and utricle. In the avian cochlea GATA3 is expressed throughout the sensory epithelia [SE]. However, expression is limited to the striola of the utricle. The striola corresponds to an abrupt change in morphologically distinct hair cell types and a 180 shift in hair cell orientation. To identify genes co-expressed with GATA3 in the utricle striola, we compared gene expression differences between the SE of the striola and the surrounding region of the extra-striola on a custom transcription factor microarray. We also gene expression profiled GATA3 siRNA knockdowns and GATA3 over-expression in avian utricle SE and confirmed several of the observed gene expression differences by RNA *in-situ*. We identified 27 known modulators of Wnt signaling, specifically numerous inhibitors of Wnt signaling were up-regulated in the striola and when GATA3 was over-expressed. These were down-regulated in GATA3 knockdowns. We also identified 12 FGF signaling genes and many known regulators of axonal guidance/ neurogenesis that are specific to the striola region and differentially regulated in response to GATA3 expression levels. To determine if the differentially expressed genes are directly regulated by GATA3, we performed ChIP in dissociated SE primary cells and identified GATA3 binding sites upstream of the LMO4 and muscleblind like 2 (MBNL2) genes. Together this evidence suggests that GATA3 plays an important role in local inhibition of Wnt signaling at the striola and that this process may be involved in specifying distinct sensory hair cell types. These observations will play an important role in understanding GATA3 regulation in the inner ear and its affects on human hearing disorders.

Individuation of new mutations in L1CAM gene in patients with L1 diseases. *F. Boaretto[1], C. Bertolin[1], G. Vazza[1], L. Garavelli[4], E. Della Giustina[2], M. T. Divizia[3], A. Vettori[1], M. L. Mostacciuolo[1]* 1) Dept. Biology, University of Padova, Italy; 2) Dept. of Neuropsychiatry, Hospital of Reggio Emilia, Reggio Emilia, Italy; 3) Gaslini Institute, University of Genova, Genova, Italy; 4) Dept. of Pediatrics, Hospital of Reggio Emilia, Reggio Emilia, Italy.

Backgroung: L1CAM (Neural cell adhesion molecule L1) is a gene highly conserved in mammals and in other species, suggesting an important functional role for this adhesion molecule. Mutations of L1CAM are responsible for X-linked syndromes, including different diseases that can be associated with CNS malformations. The most frequent is the Hydrocephalus secondary to Stenosis of the Aqueduct of Sylvius (HSAS, MIM 307000) but also a severe condition such as MASA (Mental retardation, Aphasia, Shuffling gait, and Adducted thumbs. MIM 307000) has been described. Materials and methods: We identified a sample of subjects with specific severe phenotype characterized by following major features: hydrocephalus, mental retardation, spasticity of the legs, and adducted thumbs. By direct sequencing, these patients have been investigated for mutations in the coding regions of the L1CAM gene. Results: Three new mutations have been detected in three uncorrelated patients. The first one is a novel missense mutation within exon 1 (c.25T_A), leading to an amino acid substitution (p.W9R) in codon 9. The other two nucleotidic changes are frameshift mutations (c.670delC, c.2410insA) resulting in premature stop codons respectively in exons 6 (codon 224) and exon 18 (codon 804). Interestingly both the identified mutations are located in the extracellular portion of the mature protein, which is involved in homo- and heterophilic protein-protein interactions. Conclusions: MASA syndrome is an extremely rare, severe condition associated with both missense and frameshift mutations of L1CAM gene.

Characterization of Neuronal Storage in the Mucolipidosis Type IV Murine Model. *C. Curcio-Morelli[1], B. Venugopal[1], M. F. Browning[1], J. Pickel[2], S. U. Walkley[3], S. A. Slaugenhaupt[1]* 1) Center for Human Genetic Research, Harvard Medical School, Boston, MA; 2) NIMH Transgenic Core, National Institutes of Health, Bethesda, MD; 3) Dominck P. Purpura, Albert Einstein College of Medicine, Bronx, NY.

We have recently reported the first murine model for MLIV, which accurately models most phenotypes seen in MLIV patients. Here we characterized the cellular storage phenotype in Mcoln1-/- mice in both fibroblasts and embryonic neuronal primary cultures. Fibroblasts established from both Mcoln1-/- embryos and post-natal day four pups showed a defect in trafficking after incubation with BODIPY- FL ceramide, as previously described in human MLIV fibroblasts. Analysis of fluid-phase endocytosis showed an increase in the number and size of FITC-dextran-labeled structures in Mcoln1-/- fibroblasts when compared with Mcoln1+/+, suggesting that the endocytosed material is accumulated and/or fluid phase endocytosis is increased in MLIV. The availability of the Mcoln1-/- mouse model allows, for the first time, characterization of cellular phenotypes in neurons. The Mcoln1-/- mice present with numerous dense inclusion bodies in all cell types in brain and particularly in neurons. Mcoln1-/- embryonic E15 neuronal primary cultures (E15NPC) revealed intense staining of acidic vesicles after lysotracker staining. Electron microscopy of E15NPC showed the presence of several concentric membranes in the cytoplasm (zebra bodies). Recently it was suggested that the storage in human MLIV fibroblasts could be rescued with low-dose chloroquine treatment, and therefore we investigated the effects of chloroquine on Mcoln1-/- neuronal cells. E15NPC were treated with 10 nM chloroquine for 4 days and lysotracker staining showed an increased number of acidic vesicles in Mcoln1-/- after treatment. Electron microscopy revealed enlarged, multi-vesiculated storage bodies, suggesting that chloroquine is unlikely to resolve neuronal storage. The presence of storage bodies in neurons established from embryonic stages of MLIV mice provides us with an excellent tool to test strategies and potential drugs aimed at ameliorating this neurological disorder.

The mitochondrial protease AFG3L2, a key player in axonal development and cerebellar degeneration. *G. Casari[1], F. Maltecca[1], L. Cassina[1], G. A. Cox[2], J. L. Guenet[3], A. Quattrini[1]* 1) Dept Dibit, Vita-Salute San Raffaele Univ, Milan, Italy; 2) The Jackson Laboratory, 600 Main Street, Bar Harbor, ME 04609, USA; 3) Institut Pasteur, 25 Rue Du Docteur Roux, 75724 Paris CEDEX 15, France.

Axonal degeneration of the longest motor and sensory axons is the main pathological feature of hereditary spastic paraplegia (HSP). Mutations of paraplegin, a nuclear-encoded mitochondrial metalloprotease, cause a recessive form of HSP. We showed that paraplegin co-assembles with a highly homologous protein, AFG3L2, to form a functional complex (the m-AAA protease) in the inner mitochondrial membrane. Lack of this complex in HSP primary fibroblasts causes a reduced complex I activity and an increased sensitivity to oxidative stress. The paraplegin mouse model partially recapitulates what observed in patients, showing a slowly progressive axonal degeneration. We are characterizing two different mutant mouse models defective in Afg3l2. In spite of the close functional association of AFG3L2 with paraplegin, either Afg3l2 mutation leads to a more severe neurological syndrome. Actually, both Afg3l2 mutants show a dramatic neuromuscular phenotype beginning at P7 with hindlimbs paraparesis which progresses until complete tetraparesis and death, generally at P16-18. Morphological analysis shows a marked impairment of axonal development with delayed myelination and poor axonal radial growth. The presence of morphologically altered mitochondria and the highly reduced enzymatic activities of the respiratory chain denote the mitochondrial origin of the disease. Moreover, Afg3l2 mutation severely affects cerebellum, which is spared in paraplegin-deficient mouse. Interestingly, the critical region associated to the recently reported spinocerebellar ataxia type 28 locus (SCA28) includes few neural genes and, among these, AFG3L2. This evidence, together with the cerebellar phenotype of the Afg3l2 mutant mice, points to AFG3L2 as the excellent candidate for this disease.

Genome-wide association for cognitive ability and overlap with results from Alzheimers disease. *Q.-R. Liu[1], T. Drgon[1], C. Johnson[1], P.-W. Zhang[1], D. Walther[1], J. Hess[2], K. Bolla[3], J. L. Cadet[4], C.-Y. Li[1,5], M. Nino[1], G. R. Uhl[1]* 1) Molecular Neurobiology Branch, NIDA/NIH, Baltimore, MD; 2) Office of the Clinical Director, NIH-IRP (NIDA), 333 Cassell Drive, Suite 3510, Baltimore MD 21224; 3) Department of Neurology, Johns Hopkins Bayview Medical Center, Baltimore MD 21224; 4) Molecular Neuropsychiatry Branch, NIH-IRP (NIDA), Baltimore MD 21224; 5) Center for Bioinformatics, National Laboratory of Protein Engineering and Plant Genetic Engineering, College of Life Sciences, Peking University. Beijing, China 100871.

Classical genetics documents substantial heritable components that underlie individual differences in human cognitive abilities. However, only little information documents which gene variants are likely to contribute to these individual differences or the ways in which these differences could contribute to interactions between cognitive abilities and vulnerabilities to Alzheimers disease (AD). We have used genome-wide association (GWA) with 1M SNP arrays to seek haplotypes that could contribute to individual differences in an assessment of general cognitive ability in each of three samples of research volunteers. We have also sought convergence of these results with data from two previously reported GWA studies of AD. We have identified replicable results from the three cognitive function samples that converge much more than expected by chance. These results identify cognitive function genes in a number of classes, including many whose allelic variants are likely to influence neuronal connectivities. Results from each of two reported AD GWA studies also converge much more than expected by chance. Findings are generalizable, since the cognitive function results fit remarkably well with those for AD. Effects at each individual gene locus are modest. Nevertheless, the data provide strong molecular genetic support for classical genetic results concerning the heritabilities of cognitive abilities and AD and novel support for shared genetic influences on both phenotypes. The data add to evidence that variants in genes that alter neuronal connectivities influence a number of brain phenotypes and disorders.

Pharmacological chaperone treatment for Pompe disease. *J. J. Flanagan, H. V. Do, X. Wu, A. C. Powe, R. Khanna, B. Ranes, K. Tang, C. Pine, H. Williams, R. Soska, L. Pellegrino, J. Feng, M. Zdancewicz, E. R. Benjamin, B. A. Wustman, K. J. Valenzano, D. J. Lockhart* Amicus Therapeutics, Cranbury, NJ.

Pompe disease is caused by deficient acid alpha glucosidase (GAA) activity which impairs lysosomal glycogen metabolism. The enzyme deficiency leads to lysosomal glycogen accumulation and results in progressive skeletal muscle weakness, reduced cardiac function, respiratory insufficiency, and CNS impairment at late stages of disease. Genetic mutations in the GAA gene result in either lower expression or produce mutant forms of the enzyme with altered stability, and/or biological activity ultimately leading to disease. Pharmacological chaperones represent a promising new therapeutic approach for the treatment of genetic diseases. In this study, we show that the pharmacological chaperone AT2220 (1-deoxynojirimycin-HCl) binds to mutant GAA and increases its stability. In Pompe patient-derived fibroblasts and in transiently transfected COS-7 cells expressing certain GAA missense mutations, AT2220 significantly increases GAA levels. AT2220 also increased GAA levels in disease-relevant tissues of wild-type mice, rats and monkeys suggesting that AT2220 may be appropriate for Pompe patients with the common IVS 1 (-13 T>G) splicing defect which produces low levels of wild-type GAA. In cell lines derived from late-onset Pompe patients with this common splicing mutation, AT2220 increased GAA levels. We are also investigating the ability of AT2220 to improve the biological properties of enzyme replacement therapy. In rats, the plasma half-life of recombinant human GAA (rhGAA) increased 2-fold when AT2220 (30 mg/kg p.o.) was administered 30 minutes prior to rhGAA injection. In GAA KO mice, the uptake of rhGAA was increased approximately 2-fold in heart and diaphragm when AT2220 (100 mg/kg p.o.) was administered prior to rhGAA injection. These data indicate that co-administration of a pharmacological chaperone with rhGAA may increase the enzymes exposure and tissue uptake in vivo.

Genome-wide association scan for genetic determinants of warfarin dose. *R. McGinnis[1], F. Takeuchi[1], S. Bourgeois[1], N. Soranzo[1], V. Ranganath[1], N. Eriksson[2], J. Lindh[3], A. Rane[3], M. Wadelius[2], P. Deloukas[1]* 1) Wellcome Trust Sanger Institute, Cambridge, United Kingdom; 2) Department of Medical Sciences, Clinical Pharmacology, University Hospital, Uppsala, Sweden; 3) Clinical Pharmacology, Karolinska Institute, Campus Huddinge, Stockholm, Sweden.

Warfarin is an anticoagulant that is the most widely prescribed therapy for reducing thromboembolic events that often give rise to stroke, deep vein thrombosis, pulmonary embolism or serious coronary malfunctions. A combination of genetic and non-genetic factors cause Caucasians to exhibit 10-fold interindividual variation in required dose (RD) needed for the usual therapeutic level of anticoagulation (2.0-3.0 PT INR). Thus, in the absence of information (genotypic, clinical, etc.) for predicting each patient's RD, initial prescribed doses may be too low (risking failure to protect the patient) or too high (risking over-anticoagulation and severe bleeding). We were among the first to show that polymorphism in the warfarin drug target (VKORC1) accounts for a major portion (~30%) of the variance in RD and have recently evaluated 1523 Swedish patients from the Warfarin Genetics (WARG) cohort in the largest study to date showing likely patient benefit from genetic forecasting of RD (Wadelius et al. Blood, in press). This study confirmed that SNPs in VKORC1 and CYP2C9 predict at least 40% of RD variance while non-genetic factors (age, gender, etc.) jointly account for another ~15%. We are now searching for additional genetic predictors of RD in a large-scale genome-wide association study (GWAS) of 370,000 SNPs (Illumina 370K) genotyped in >1,000 WARG patients. Our initial GWAS results based on both single marker and haplotype analysis identified CYP4F2 as at least one additional genetic predictor of RD and we have replicated this finding in a further 600 samples (combined p-value of 8.9×10^{-10}). We will show that detection of additional genetic predictors critically depends on the statistical technique applied, and will also report on the analysis of CNVs and on additional warfarin-related phenotypes (e.g. over-anticoagulation).

Variants in Neuroligin Pathway Genes and Autism Spectrum Disorder. *K. Steinberg[1,2], M. Zwick[1]* 1) Department of Human Genetics, Emory University School of Medicine, Atlanta, GA; 2) Graduate Program in Population Biology, Ecology and Evolution, Emory University, Atlanta, GA.

Neuroligins are cell adhesion molecules important in the post-synaptic density. Recent studies demonstrate that mutations in the X linked genes neuroligin 3 (NLGN3) and 4X (NLGN4X) contribute to autism spectrum disorder (ASD). However other studies failed to find these associations in individuals with ASD. To comprehensively survey the common and rare variation in these regions, we are resequencing NLGN3 and NLGN4X in individuals from families with two or more affected male sibpairs from the Autism Genetic Resource Exchange (AGRE). Affected male sibpairs were chosen based upon sharing identical markers near NLGN4X (DXS9895 and 9902). One male from each sibpair was selected for resequencing. The fathers of the affected sibpair were selected as unaffected controls. Sequence data from the 101 affected males and their matched controls will be presented. Target DNA, isolated with Microarray-based Genomic Selection (MGS), was hybridized to a custom designed high density resequencing array containing unique coding and non-coding sequences from NLGN3 and NLGN4X, and coding sequences from neurexin-1beta (NRXN1b) and SHANK3. A total of 59.5 Mb of sequence was generated with an average conformance of 92.6% and average call rate of 90.9%. SNPs identified in our study are partitioned into functional classes (UTR, silent, replacement, intron, intergenic) and compared within and between these classes. We have identified 136 replacement SNPs that have been evaluated for evolutionary conservation using the phastCon program, severity of functional mutations using the SIFT program and presence or absence from dbSNP. We are currently in the process of validating these SNPs using traditional Sanger sequencing and will present the results of our analysis.

Clinical, Biochemical and Molecular Characterization of a Family with Hepatoerythropoetic Porphyria. *J. L. Cohen[1], L. Liu[1], D. Doheny[1], J. L. Cantatore-Francis[2], J. V. Schaffer[2], R. J. Desnick[1], M. Balwani[1]* 1) Dept. Genetics and Genomic Sciences, Mount Sinai School of Medicine, New York; 2) Dept. Dermatology, NYU School of Medicine, New York.

Porphyria Cutanea Tarda (PCT) is an autosomal dominant inborn error of heme biosynthesis which results from decreased hepatic uroporphyrin decarboxylase (UROD) activity (<30% of nl). A familial form is caused by heterozygous mutations in UROD, while Hepatoerythropoietic Porphyria (HEP) is the very rare autosomal recessive form of PCT. We report a non-consanguineous family of Puerto Rican/Dominican descent, in which both parents and two children have PCT, and three have HEP of varying severity. The proband, a 7 year old girl, presented with photosensitivity and blistering/scarring lesions on the dorsum of hands, skin fragility, hypertrichosis of the face, and onycholysis. She had anemia and dark colored urine, but denied abdominal pain or neurologic symptoms. Two symptomatic siblings had similar manifestations. Lab studies showed normocytic anemia, increased urine total porphyrins >6,000 (nl 0-300) nmol/24h (primarily uroporphyrin and heptacarboxylporphyrin), and normal ALA and PBG levels. Plasma total porphyrins and protoporphyrins were elevated with fluorescence at 619 nm, suggestive of HEP. Erythrocyte UROD activity was markedly decreased. Similar, but milder findings were noted in the other affected siblings. UROD sequencing of the probands genomic DNA revealed a deletion, 645del1053ins10, and a novel missense mutation, V166A. The mother had the deletion, the father the missense mutation. The two asymptomatic siblings were heterozygous for V166A. HEP has clinical manifestations similar to Congenital Erythropoetic Porphyria, including skin lesions, splenomegaly, and hemolytic anemia. Sun protection is the key to avoidance of severe scarring. Therapeutic options are limited and the long term natural history of this rare disorder is unknown. The three affected HEP children with the same genotype varied phenotypically, having mild to severe cutaneous involvement, anemia, and accumulated porphyrin levels, emphasizing the variable intra-familial expression in this rare disorder.

Polymorphisms in the promoter region of Nrf2 and association with systemic lupus erythematosus, juvenile, rheumatoid arthritis and asthma. *E. J. Cordova[1], V. Baca[2], J. Ramírez[1], S. Jiménez[1], R. Velázquez[1], F. Centeno[1], L. Orozco[1]* 1) Investigación, Instituto Nacional de Medicina Genómica, Mexico D.F; 2) Centro Médico Nacional, Siglo XXI, IMSS.

The Nrf2-Keap1 pathway is one of the main mechanisms against xenobiotics and oxidative stress. The Nrf2 transcription factor is required for both basal and inducible expression of a number of genes encoding antioxidant, detoxificant and immune associated enzymes. Expression of Nrf2-dependent genes confers cellular protection from different environmental insults. Murine models have highlighted the participation of Nrf2 in systemic lupus erythematosus and asthma. The -653G/A and -617C/A SNPs, in the 5' regulatory region of NRF2 gene, have been associated with an increased risk for gastric mucosal inflammation, acute lung injury and aberrant DNA methylation in gastric epithelium. The aim of this study is to determine whether NRF2 SNPs are associated with systemic lupus erythematosus (SLE), juvenile rheumatoid arthritis (JRA) and asthma. Three hundred patients with SLE, 250 with asthma and 129 with JRA were enrolled into the study. In addition 300 unrelated healthy subjects without immune and inflammatory diseases were also included. All the subjects were recruited from four tertiary level Institutions from Mexico City. Allelic discrimination was performed by TaqMan assay and Fluorescent Fragment Analysis. The association test, Hardy-Weinberg equilibrium (H-WE) and haplotypes were determined using EPIDAT, FINETTI and Haploview softwares, respectively. Genotype distribution in cases and controls were in H-WE. Our results did no show evidence of association of NRF2 gene with any of the three studied diseases. However, when we explored the possible association between these SNPs with lupus nephritis, we could observe that the frequency of heterocygote -650C/A SNP was significantly lower in SLE female cases without nephritis than those with nephritis. Interestingly, our results suggest that in Mexican population this gene confers suceptibility to develop lupus nephritis and it seems that it is in a gender-dependent manner.

Outcome of genetic test result review in the DuchenneConnect Registry. *V. Rangel Miller[1], W. A. Faucett[1], K. Loud[1], M. Hegde[1], G. Spinella[2], P. Furlong[2]* 1) Dept of Human Genetics, Emory University, Decatur, GA; 2) Parent Project Muscular Dystrophy, Middletown, OH.

Purpose: In Duchenne/Becker muscular dystrophy (DBMD), well-characterized mutations are necessary to evaluate eligibility for therapeutic trials using molecular genetic corrective approaches. Despite advances in genetic testing, many patients do not have results that provide adequate mutation information. DuchenneConnect is a self-report patient registry, developed to connect the patient, provider and research communities and to facilitate participation in research opportunities. We present the frequencies of genetic testing, informative test results and the need for further testing among registry participants. We emphasize the availability of registry data to researchers for use in prospective study design, feasibility planning and patient recruitment. **Methods:** We developed a web-based survey stored in a HIPAA-compliant database to capture patients clinical presentation and *DMD* mutation. Molecular test results are reviewed by trained genetic counselors and geneticists and compared to the Leiden database. Design of the survey and review of the data follows guidelines employed by other North American DBMD registries and the TREAT-NMD Neuromuscular Network. **Results:** Of 970 participants to date, 507 completed a survey of which 84% had genetic testing, 8% had a muscle biopsy alone, 6% did not have testing and 2% did not know. From preliminary test result curation, 69% were informative, 20% warranted further mutation characterization and 11% had research results that needed clinical laboratory confirmation. In total, 42% of participants were eligible for additional testing. **Conclusion:** New therapies are being developed utilizing specific molecular genetic corrective approaches. A substantial number of participants do not have well characterized mutations and will need additional molecular testing to determine their eligibility for clinical trials. The improved mutation characterization of participants in the registry will provide critical data for researchers to identify potential participants and obtain data for study design and planning.

A submicroscopic tandem duplication in 6p21.2, including *KCNK16* and *KCNK17*, in a 10-month-old girl with an intractable epilepsy. *M. Nesteruk[1,2], B. C. Lanpher[3], Z. Xia[1], S. W. Cheung[1], P. Stankiewicz[1]* 1) Dept. of Molecular & Human Genetics, Baylor College of Medicine, Houston, TX; 2) Dept. of Medical Genetics, Institute of Mother and Child, Warsaw, Poland; 3) Vanderbilt Children's Hospital, Nashville TN.

Recent studies have indicated a genetic contribution to epilepsy in ~40% patients. Seizures can be inherited in a Mendelian, non-Mendelian, or complex trait fashion. In the majority of identified cases, the pathogenic mutations have been found in genes encoding subunits of ion channels. We present a 10-month-old girl with intractable epilepsy, including infantile spasms, in whom, using a clinical targeted oligonucleotide array-CGH V6.5 (Agilent, 44K), we identified a submicroscopic duplication in 6p21.2, involving three potassium channel genes: *KCNK5*, *KCNK16*, and *KCNK17*. The same duplication was detected in the patients mother, who as a child had delayed motor development with no episodes of epilepsy. Interestingly, Sáez-Hernández et al. (2003) reported a balanced translocation t(4;6)(q35;p21) with a 6p21 breakpoint mapping 3.5 kb upstream to *KCNK16* in two sisters and their mother, wherein only the sisters manifested the idiopathic generalized epilepsy. Whole-genome array CGH analysis with ~385,000 oligonucleotide probes (NimbleGen) allowed breakpoint sequencing with help of long range PCR. The duplication is 280,796 bp in size, tandem in orientation, and contains entire *KCNK16* and *KCNK17* and exon 1 of *KCNK5*. We found a 5.6 kb LINE-1 retrotransposon element L1PA11 and six nucleotide insertion at the junction fragment, indicating a nonhomologous end joining (NHEJ) mechanism of formation. Supporting this, recently, Korbel et al. (2007) and Kidd et al. (2008) identified retrotransposons in one third of the CNV breakpoints. We suggest that the epilepsy in our patient is due to an increased dosage of *KCNK16* that could be imprinted, similarly as another recently reported member of the potassium channel family, *KCNK9*.

Identification of copy number variants implicated in the development of neural tube defects. *K. Soldano[1], A. Dellinger[1], D. S. Stamm[2], A. Trott[1], N. Ellis[1], D. G. Siegel[1], H. Cope[1], P. Xu[1], C. F. Potocky[1], C. S. Haynes[1], T. M. George[3], A. E. Ashley-Koch[1], S. G. Gregory[1]* 1) Center for Human Genetics, Duke University Medical Center, Durham, NC; 2) University of California, Davis School of Medicine, Sacramento, CA; 3) Dell Children's Medical Center of Central Texas, Austin, TX.

Neural tube defects (NTDs) are among the most serious congenital birth defects and result from the neural tube failing to close during the first three to four weeks of fetal development. NTDs are a phenotypically heterogeneous group of disorders with a large spectrum of clinical presentation and degree of impairment. The most common NTD presentations are lumbosacral myelomenginocele (also known as spina bifida) and anencephaly. In the US, spina bifida and anencephaly occurs in 19.6 per 100,000 live births and 10.4 per 100,000 live births, respectively. Non-syndromic NTDs comprise the majority of NTDs, and are thought to have a multifactorial etiology with a complex interplay of genetic and environmental factors. We recently completed a genome wide association screen (GWAS) of 50 families affected with cranial NTDs using the Illumina Infinium HumanHap300 genotyping microarray. As part of this analysis we used the GWAS data to identify copy number variants (CNVs), genomic deletions or duplications, which may harbor genes that contribute to NTDs. This was achieved using Nexus Copy Number software (BioDiscovery, Inc.). The most interesting findings amongst the detailed analysis, consisting of 49 anencephaly affected individuals, 2 affected siblings, 36 unaffected siblings and 50 parents, were de novo rearrangements containing ERBB4 and CNTNAP2, and familial CNVs that were independent of known CNVs in the AKT3 gene. Genomic rearrangements were also detected in HDAC9, FRG2C, and the KIR gene cluster although those genomic regions contain known CNVs. AKT3, CNTNAP2, HDAC9 and ERBB4 could be relevant to the etiology of NTDs because they are expressed in the fetal brain and may play a role in the developing neural tube.

Association between GP6 polymorphisms and coronary heart disease in a community-based population: The Western New York Acute Myocardial Infarction Study. *J. Shaffer[1], C. Kammerer[1], L. Iacoviello[2], M. Trevisan[2], R. Ferrell[1], R. Donahue[2]* 1) U Pittsburgh, Pittsburgh, PA; 2) U Buffalo, Buffalo, NY.

Chronic inflammation, collagen-induced platelet aggration, and endothelial dysfunction are risk factors for acute myocardial infarction (MI). The genetic contributions to cardiac disease that involve these and other pathways have been understudied, especially in community-based samples. From 1995-2001, The Western New York Acute MI Study identified survivors of acute MI from hospitals in Erie and Niagara Counties and matched them with an equal number of apparently healthy community-based controls on sex and age (1 year). 756 cases and 751 controls (comprising 1168 men aged 35-70 and 339 post-menopausal women) were genotyped for 31 functional single nucleotide polymorphisms (SNPs) in 24 loci involved in inflammation, collagen content, and endothelial function pathways. Using logistic regression, while simultaneously adjusting for several covariates (age, sex, body mass index, hypertension, high cholesterol, smoking status, diabetes, and family history of heart disease), we tested for an association between each SNP and MI status. A missense SNP (minor allele frequency [MAF]=0.16) in GP6, a glycoprotein receptor for collagen found in platelets, was associated with MI after permutation-based adjustment for multiple testing (OR=0.70; p=0.002; adjusted p=0.068). We further investigated the role of GP6 in risk for MI by genotyping and testing 4 additional intronic SNPs spanning the GP6 gene, all of which are in low linkage disequilibrium (r^2=0.00 to 0.51) with the missense SNP and with each other. One of these additional GP6 SNPs was also associated with MI (OR=0.75; p=0.004; MAF=0.22). Haplotype analysis of GP6, while confirming the SNP-wise associations, did not yield evidence that any specific haplotype(s) accounts for the observed associations. Overall, these findings suggest genetic variants in GP6 may predispose for MI through its activating role in platelet aggregation. Identification of the genetic risk factors for MI may lead to a better understanding of disease etiology and possible therapeutic interventions for cardiac disease.

Kinetin treatment in transgenic mice carrying mutant human *IKBKAP*: A model for treating human splicing disease. *R. S. Shetty[1, 2], Y. T. Chen[1, 2], M. M. Hims[1, 2], J. Mull[1, 2], M. Leyne[1, 2], L. Liu[1, 2], J. Pickel[3], S. A. Slaugenhaupt[1, 2]* 1) Center for Human Genetic Research, MGH , Boston, MA; 2) Harvard Medical School, Boston, MA; 3) National Institute of Mental Health, National Institutes of Health, Bethesda, MD.

The study of human genetic disease highlights the importance of mRNA splicing, as 20-30% of all disease causing mutations result in splicing defects. The plasticity of the splicing reaction has made these mutations attractive candidates for the development of therapeutics, since a slight shift in isoform ratios can lead to dramatic phenotypic improvement. In Familial Dysautonomia (FD), a severe neurodegenerative disorder, all patients have an intronic splice site mutation (IVS20+6T>C) in the *IKBKAP* gene (FD *IKBKAP*), which results in tissue-specific skipping of exon 20. Although FD is a recessive disease, homozygous mutant cells express wild-type *IKBKAP* transcripts, and produce normal IKAP protein. The relative amount of wild-type and mutant *IKBKAP* mRNAs varies between tissues, with the lowest levels of wild-type *IKBKAP* production in tissues from the nervous system. Recently, we created transgenic mouse lines using BACs containing human *IKBKAP* carrying the same intronic splice mutation present in all FD patients. These humanized FD *IKBKAP* transgenic mice exhibit the same tissue-specific aberrant splicing pattern of as the FD patients. We have previously shown that plant cytokinin kinetin can significantly improve the production of normal *IKBKAP* transcripts in FD lymphoblast cell lines. In this study we tested the ability of kinetin to alter *IKBKAP* splicing in vivo by treating transgenic mice by oral gavage or dietary supplementation. Treatment with kinetin modifies splicing in all tissues assayed in the FD *IKBKAP* transgenic mice, including brain. These exciting results prove that dietary supplementation with kinetin holds great promise as a potential therapeutic for FD.

Exhaustive search for two-locus interactions in genome-wide association study of Crohns disease. *T. Bhangale, L. Cardon* Human Biology, Fred Hutchinson Cancer Research Center, Seattle, WA.

Simulation studies suggest that searches for two locus interactions (TLI) using regression models are computationally feasible for the large-scale datasets involved in genome-wide association studies (GWAS) and can be more powerful than single locus approaches. While an exhaustive search for TLI using a genome-wide dataset is possible, it involves several computational challenges: the time required to perform billions of tests and memory and storage requirements. Results of such an analysis have so far not been reported. We developed a computer program that uses parallel computing and data compression methods to overcome these difficulties to analyze GWAS datasets for all TLI by performing a likelihood ratio test for the fit of the full logistic regression model (up to 9 parameters). We apply the method to Wellcome Trust Case Control Consortiums Crohns disease data. Since the test will detect main and/or interaction effects, even after applying the conservative Bonferroni correction, 3983679 pairs showed significant association. We analyzed these pairs further to find those that showed interactions over and above the main effects and discovered 17 new such potential TLI. We verify the previous simulation-based findings that two-stage approaches that only test for interactions among the SNPs with large marginal effects, are not as powerful as an exhaustive search. We find that an association study for TLI faces new difficulties e. g. possible failure of imputation methods at interacting loci due to their reliance on reference haplotypes (similar to control haplotypes) as TLI imply a difference in LD between the interacting loci in cases and controls; poorly understood genotyping artifacts that lead to false positive TLI despite clear clusters on the scatter plots of signal intensities for the two alleles; and difficulties in performing permutation tests. It is likely that current GWAS, which are mainly focused on the marginal effects of SNPs, will soon be followed by scans for TLI. In this work we provide one possible framework for doing such analysis and illustrate the type of results and quality control issues that are likely to be encountered.

Trisomy X in dogs, humans, and other species. *U. Prociuk[1], V. Meyers-Wallen[2], M. E. Haskins[1,3], M. L. Casal[1]* 1) Section of Medical Genetics, Veterinary Hospital Univ of PA, Philadelphia, PA; 2) Dept. of Biomedical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY; 3) Dept.of Pathobiology, School of Veterinary Medicine Univ of PA, Philadelphia, PA.

First described as a super female Trisomy-X is one of the most common chromosomal sex abnormalities seen in humans. This syndrome is a form of chromosomal variation characterized by the presence of an extra X chromosome which occurs in one of every 1002 newborn girls. Most women with Trisomy X are asymptomatic and thus indistinguishable from normal XX females unless karyotyping is performed. While this syndrome rarely causes any unusual physical features or medical problems in humans, females with trisomy X may have low birth weight, menstrual irregularities, are taller than average and rarely exhibit severe mental impairments with an increased risk of learning disabilities, delayed speech and language skills. Trisomy X females have normal sexual development and are able to conceive children. Trisomy X has been recognized in a variety of other species animals such as dog, cat, cattle and horse, mice and river buffalo. As in humans, most animal species presented here were essentially normal on physical examination with the exception of abnormal external genitalia in one dog and small stature in a cat and one cow. Delayed puberty, primary amenorrhea/primary anestrus, and irregular cycles have been described in all affected species including humans. A common finding in both humans and animals is the presence of ovarian dysgenesis and infertility, which was the main reason for cytogenetic studies. Previously, only three cases of trisomy X have been described in dogs with persistent anoestrus. We have identified and present here two cycling dogs that were examined for infertility in which karyotyping revealed trisomy X. Many women and some animals with trisomy X cycle and can conceive. As the incidence of trisomy X is fairly high in humans, it is likely underdiagnosed in animals underscoring the value of karyotyping in companion animals.

Polymorphisms in the MYH9 gene are associated with non-diabetic ESRD in African Americans. *M. A. Bostrom[1], P. J. Hicks[1], M. E. Cunningham[2], J. Divers[2], J. B. Kopp[3], C. A. Winkler[4], G. W. Nelson[4], C. D. Langefeld[2], D. W. Bowden[1,5,6], B. I. Freedman[5]* 1) Dept Biochemistry, Wake Forest Univ, Winston-Salem, NC; 2) Biostatistical Sciences, Wake Forest Univ, Winston-Salem, NC; 3) Kidney Disease Section of the NIDDK, Bethesda, MD; 4) SAIC National Cancer Institute-Frederick, Frederick, MD; 5) Internal Medicine/Nephrology, Wake Forest Univ, Winston-Salem, NC; 6) Center for Human Genomics, Wake Forest Univ, Winston-Salem, NC.

African Americans (AA) have increased susceptibility to non-diabetic etiologies of end-stage renal disease (ESRD), particularly attributed to hypertension and glomerular disease, relative to European Americans. In AA the non-muscle myosin heavy chain 9 (MYH9) gene is strongly associated with focal and segmental glomerulosclerosis and HIV-associated nephropathy using Mapping by Admixture Linkage Disequilibrium (MALD); and a four SNP haplotype (E1) in this gene (3224; rs4821480, rs2032487, rs4821481, and rs3752462) is significantly associated with both kidney diseases. This haplotype has also been shown to be associated with non-diabetic etiologies of ESRD (not diabetic ESRD) in limited numbers of AA patients. We attempted to replicate this genetic association in 1817 AA born in the southeastern U.S. (871 with non-diabetic ESRD and 946 controls lacking nephropathy). Fifteen SNPs were genotyped and a single SNP (rs2187776) deviated from Hardy-Weinberg Equilibrium in controls and was excluded. Thirteen remaining SNPs were significantly associated with non-diabetic ESRD (recessive model; $p = 0.01 - 4.0 \times 10^{-22}$; odds ratio (OR) = 1.38-2.96). The four SNP E1 haplotype (3224) was associated with non-diabetic ESRD under the recessive model ($p = 1.56 \times 10^{-20}$; OR = 2.2; confidence interval (CI) = 1.82 - 2.68). After adjusting for this haplotype, logistic regression analysis showed that 5 SNPs remained associated with non-diabetic ESRD, rs12107, rs5756152, rs1005570, rs16996674, and rs16996677 (additive model; $p = 0.02 - 9.08 \times 10^{-7}$; OR = 1.22-1.50). We conclude that the 4 SNP E1 haplotype is associated with increased risk of non-diabetic ESRD among AA and potentially additional variants in MYH9 contribute to non-diabetic etiologies of ESRD.

Population-Based GWAS Reveals Four Novel Trans-Acting Loci Influencing Plasma Levels of Liver Enzymes. *X. Yuan[1], D. Waterworth[1], J. Perry[2], N. Lim[1], K. Song[1], J. Chambers[3], W. Zhang[3], P. Vollenweider[4], G. Waeber[4], L. Cardon[1], T. Frayling[2], J. Kooner[5], V. Mooser[1]* 1) Genetics Division, GlaxoSmithKline, Collegeville, PA; 2) Genetics of Complex Traits, Institute of Biomedical and Clinical Sciences, Peninsula College of Medicine and Dentistry, University of Exeter, UK; 3) Department of Epidemiology and Public Health, Imperial College London, UK; 4) Department of Medicine and 5National Heart and Lung Institute, Imperial College London, UK; 5) National Heart and Lung Institute, Imperial College London, UK.

BACKGROUND : Plasma levels of liver enzymes (PLEs) are widely used for the diagnosis of liver diseases and to follow the response to treatments. PLEs levels are known to be heritable. An understanding of the molecular determinants of PLEs is of paramount importance for a proper interpretation of liver laboratory tests and to expand our knowledge of liver diseases. METHOD : We conducted a GWAS analysis on Alanine-Aminotransaminase (ALT), Alkaline phosphatase (ALP), Gamma glutaryl transferase (GGT) and Aspartate aminotransferase (AST) considered as quantitative traits in three discovery and three replication cohorts with up to 12,419 individuals. Meta-analysis was conducted on the summary results from both genotyped and imputed SNPs. RESULTS : 10, 6 and 18 genotyped SNPs were replicated at genome-wide significant level ($p\ 10^{-7}$) for plasma ALT, GGT and ALP levels, respectively, whereas none was associated with AST level. Among the four newly identified trans-acting loci, the lead SNPs in PNPLA3 were strongly associated with levels of ALT ($p=8.4\times10^{-16}$) and AST ($p=5.7\times 10^{-6}$). A strong interaction with obesity was observed, suggesting that PNPLA3 might predispose to liver fat accumulation. Robust associations with cis-acting SNPs were identified for plasma GGT and ALP levels. CONCLUSION : We have discovered novel loci that influence the plasma liver enzymes levels. These discoveries shall assist in the interpretation of PLEs and may point to new mechanisms, and potentially new targets for liver diseases.

Mosaic trisomy 9: Three new cases with variations in phenotype. *B. A. Kozel[1], D. K. Grange[1], S. Kulkarni[2], T. Reimschisel[3]* 1) Genetics and Genomic Medicine, Washington Univ. School of Med., St. Louis, MO; 2) Clinical and Molecular Cytogenetics, Washington Univ. School of Med., St. Louis, MO; 3) Developmental Medicine and Cognition, Vanderbilt Univ. School of Med., Nashville, TN.

The phenotype associated with mosaic trisomy 9 is highly variable. We present 3 new patients with phenotypes of varying degrees of severity. Case 1 exhibited a more classic presentation. The phenotype included IUGR, congenital heart defect with PFO, hypoplastic aortic arch and large PDA, micropenis, respiratory failure, and hypotonia. Facial features, including hypotelorism and microophthalmia, were suggestive of holoprosencephaly, although head ultrasound was normal. Abdominal ultrasound was also normal. Amniocentesis showed 80% 47,XY,+9 cells and 20% 46,XY cells. Postnatal FISH studies of buccal cells showed 685/1000 (68.5%) trisomy 9 cells. Case 2 showed more subtle features. The infant presented at 4 months of age due to global developmental delay, bilateral congenital hip dysplasia, and Duane syndrome of the left eye. Microarray analysis of peripheral blood with confirmatory FISH revealed 26% 47,XX,+9 cells and 74% 46,XX cells. Her facial dysmorphisms include thick hair, micrognathia, and bulbous nose. She has had persistent gastroesophageal reflux and difficulty gaining weight. Echocardiogram and abdominal ultrasounds were normal. Case 3 is a boy who is now 10 years old. His peripheral blood shows 24% 47,XY,+9 cells and 76% 46,XY cells. He has mental retardation, CP, and seizures, as well as Duane syndrome. His facial features include bilateral preauricular pits, small palpebral fissures, and mild facial asymmetry. These cases demonstrate two points. First, although the degree of mosaicism is not completely predictive of phenotype, a higher percentage of trisomy 9 cells generally correlates with more severe features. Second, distinctive features in cytogenetic syndromes may point investigators to the discovery of new disease causing loci, possibly unmasked by uniparental disomy. Of note, at least one other patient with Duane syndrome, not linked to trisomy 9, has been found to have an alteration of the NELF gene on 9q34.3.

Association mapping of expression trait loci from peripheral blood CD4+ lymphocytes. *B. A. Raby[1], A. Murphy[1], J. Chu[1], V. Carey[1], B. J. Klanderman[1], S. Sylvia-Senter[1], J. Ziniti[1], C. Lange[2], S. T. Weiss[1]* 1) Channing Laboratory, Brigham & Women's Hospital, Harvard Medical School, Boston, MA; 2) Harvard School of Public Health, Boston MA.

Association studies of human gene expression promise to identify regulatory genetic variation that contributes to phenotypic diversity. Such studies performed to date have largely relied on expression data from immortalized cell lines. Herein we demonstrate the feasibility of expression-trait association testing using RNA derived from freshly harvested peripheral blood CD4+ lymphocytes from 154 asthmatics. We generated quantile-normalized gene expression profiles using Illumina HumanRef8 arrays that survey 20,589 RefSeq-curated mRNA transcripts. Genome-wide genotype data (534,290 autosomal SNP, Illumina Infinium 550K array) were available for these subjects and their parents. Family-based association testing was performed (additive model) using PBAT. We screened for cis-acting variants (within 50kb of transcripts), resulting in 511K comparisons. Significant associations for 405 genes were observed using a false discovery rate of 0.05 (corresponding to p-values ranging from 10^{-15} to 10^{-4}). Sizable SNP-specific genetic effects were observed (median locus-specific heritability of 0.171), with variants explaining more than 50% of expression trait variability in 16 genes. 18.35% of associations were also observed in a prior expression trait association study of immortalized cell lines from asthmatics (Dixon 2007), suggesting only modest generalizability of results from immortalized cells. These results highlight the feasibility of expression-trait association mapping in human populations for the identification of functional expression-related polymorphisms. Funding: U01 HL065899, P01 HL083069, R01 HL086601, K08 HL74193.

Proteomic Analysis of Platelet -Granules Using Mass Spectrometry: An Application to Gray Platelet Syndrome.

D. M. Maynard[1], M. Gunay-Aygun[1, 2], H. F. G. Heijnen[3], H. Edwards[1], J. G. White[4], W. A. Gahl[1] 1) MGB, NHGRI, NIH, Bethesda, MD, USA; 2) ORD, NIH, Bethesda, MD, USA; 3) Laboratory of Clinical Chemistry and Haematology and Cell Microscopy Centre, Univ Medical Centre, Utrecht, The Netherlands; 4) Depts of Laboratory Medicine and Pathology and Pediatrics, Univ of MN, Minneapolis, MN, USA.

A deficiency in granule-bound substances in platelets causes a group of congenital bleeding disorders known as storage pool deficiencies (SPDs). For disorders such as Gray Platelet syndrome (GPS), only the clinical and histological states have been defined. In order to understand the basic defect in this disorder, we are using proteomics and mass spectrometry (MS). Platelet -granules contain several adhesive proteins involved in hemostasis, and glycoproteins involved in inflammation, wound healing, and cell-matrix interactions. Our research represents the first effort to define the normal platelet -granule proteome using MS. We prepared a subcellular fraction (fr. 6) enriched in intact -granules from human platelet lysates using sucrose gradient ultracentrifugation. Fraction 6 proteins were separated and identified using SDS-PAGE and LC-MS/MS. We identified 219 non-redundant proteins, 44 of which appear to be newly described -granule proteins. Immuno-electron microscopy confirmed the presence of Scamp2, APLP2, ESAM and LAMA5 in platelet -granules for the first time. Recently, we analyzed fraction 6 proteins from two GPS patients and two controls. The number of peptides from soluble proteins synthesized in the megakaryocyte was markedly decreased or undetected in GPS fraction 6 compared to normal. The number of peptides from soluble proteins endocytosed into alpha granules was slightly decreased in GPS platelets compared to normal. The number of peptides from some membrane proteins was decreased in GPS while the number was approximately the same for others compared to normal. These results support the existence of ghost granules in GPS. This proteomic technology can be employed to characterize the intracellular vesicles of patients with other SPDs and other genetic disorders of organelle formation and trafficking.

Does dysregulation of the PDGFRA gene cause anomalies of the human pulmonary veins? Combining evidence from TAPVR genetics and model organisms. *S. B. Bleyl[1], Y. Saijoh[2], K. Shiota[3], S. Klewer[4], G. C. Schoenwolf[2]* 1) Dept Pediatrics, Univ Utah, Salt Lake City, UT; 2) Dept Neurobiology Anatomy,Univ Utah, Salt Lake City, UT; 3) Dept Anatomy Dev Biol, Kyoto Univ, Kyoto, Japan; 4) Dept Pediatric Cardiology, Univ Arizona, Tucson, AZ.

Total anomalous pulmonary venous return (TAPVR), is a life-threatening congenital heart defect inherited as a multifactorial trait via complex genetic and/or environmental factors. We mapped the first locus for isolated TAPVR to a 2.4 Mb interval of chromosome 4q12 by linkage and founder effect mapping, but a causative gene could not be identified by mutation analysis. While the embryology of normal heart stalk and pulmonary vein is well described, little is known about the embryogenesis or molecular pathogenesis of TAPVR. Indeed, no animal models for TAPVR have previously been reported. Here we report further mapping in the original TAPVR founder kindreds and several new extended TAPVR kindreds using 40 tag SNPs spanning a region of shared STR haplotypes. Alignment of phased haplotypes between kindreds implicates a narrow interval within the *PDGFRA-KIT* intragenic region, suggesting defective regulation of a neighboring gene(s) in the development of TAPVR. Using *in situ* hybridization in mouse and chick embryos, we found that *PDGFRA* and its ligand *PDGFA* are expressed in a temporal and spatial pattern consistent with a role heart stalk remodeling. We then used an *in ovo* function blocking assay in chick and a conditional knockout approach in mouse to knock down PDGFRA expression in the developing heart stalk and compared the morphology of the heart stalk and pulmonary veins using histological sections and 3D reconstruction. We observed that loss of PDGFRA function in both organisms can cause TAPVR, but with low penetrance (~10%) reminiscent of that observed in our human TAPVR kindreds. These animal models of TAPVR, the first reported, provide important insight into the pathogenesis of anomalous pulmonary vein development. Taken together, these data from human mapping and animal models support a role for PDGF-signaling in the normal development of the pulmonary veins, and in the pathogenesis of TAPVR.

Multi-dimensional analyses of genetic alterations in glioblastomas implicate three critical signaling pathways. *B. A. Santillan[1], L. A. Donehower[1], N. Schultz[2], J. N. Weinstein[3], L. Bull[1], C. Sander[2], D. Wheeler[1]* 1) Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX; 2) Computational Biology Center, Memorial Sloan-Kettering Cancer Center, New York, NY; 3) Department of Bioinformatics and Computational Biology, MD Anderson Cancer Center, Houston, TX.

Glioblastoma multiforme (GBM) is a grade 4 cancer arising from brain astrocytes. It is the most common and the most aggressive of the gliomas. As part of The Cancer Genome Atlas (TCGA) Project 94 tumor and matched normal samples have undergone exon sequencing (603 genes), RNA expression, and copy number analysis. Following a large-scale analysis of nonsynonymous point mutations, indels, and copy number variations in each tumor, three major pathways have arisen as significant in the genesis of GBM in humans. The observed activation of the RTK/Ras/PI-3K growth signaling pathway and the inactivation of the p53 and Rb signaling pathways suggest that the genes within these three pathways are key to oncogenesis of GBM. Of the tumors analyzed, 87% contain genomic alterations in at least one of four genes in the p53 signaling pathway. 77% of tumors contain alterations within at least one of five Rb signaling pathway genes, and 85% of tumors contain alterations in at least one of ten RTK/Ras/PI-3K pathway genes. Approximately 70% of the tumors analyzed contain an alteration in all three pathways. Consistent observation of copy number deletions and mutations in oncogenesis-inhibiting genes and copy number amplifications of oncogenenesis-promoting genes are consistent with expected oncogenic patterns. In addition, alterations within each of the p53 and Rb pathways are generally anti-correlated within each sample, suggesting that one alteration is sufficient to inactivate a pathway. These results demonstrate the efficacy of the TCGA Project in generating a more comprehensive picture of the molecular events associated with the development of an important human cancer.

Genome-Wide Association Study Identifies APOE, RELN and Other Loci Associated with Longevity. *R. Little[1], S. Kebache[1], J. Raelson[1], P. Van Eerdewegh[1], Q. Nguyen-Huu[1], G. Lepage[1], T. Fülöp[2], M. Dugas[3], H. Fournier[1], B. Paquin[1], J. Hooper[1], A. Belouchi[1], T. Keith[1]* 1) Dept Computational Biology, Genizon BioSciences, St Laurent, PQ, Canada; 2) University of Sherbrooke, Sherbrooke, QC, Canada; 3) Université Laval, Centre de recherche du CHUQ, Quebec, QC, Canada.

We recently completed a GWAS of longevity resulting in clinically relevant discoveries relating to protection against several common diseases of aging such as cardio-metabolic diseases, Alzheimers disease and cancer. The GWAS was performed using 520 cases (94 years of age or older) and 520 matched controls (18-65 years of age) from the Quebec Founder Population (QFP). Cases and controls were individually genotyped using the HumanHap 550 chip (Illumina). Regions with p-values that met the criteria for genome-wide significance based on permutation studies were identified both with the haplotype and single marker association tests, indicating that a well-powered GWAS using the QFP can be achieved with a relatively small sample size. The scope of the study was expanded through further GWA studies based on phenotypic and genetic stratification of the original patient population. The former identifies genes relevant for subsets of patients based on clinical features and the latter discovers genetic interactions, both epistatic and independent risk factors. Top candidate loci include APOE, an important regulator of lipoproteins that has consistently been associated with longevity, and the extracellular matrix protein RELN. APOE and RELN belong to the same canonical pathway. Both bind lipoprotein receptors to regulate the lamination of the neocortex and the foliation of the cerebellum. APOE has repeatedly been associated with increased risk of both cardiovascular disease and Alzheimers disease. Other loci, including centenarian-specific genes and genes epistastic to and/or independent of APOE and RELN, were discovered. Genes identified from these analyses have been used to build a GeneMap, consisting of networks of disease susceptibility genes and their biological pathways.

Meta-analyses of over 36,000 genome-wide association scans for fasting glucose and related glycaemic traits. *I. Prokopenko[1], C. Langenberg[2], J. Florez[3,4], R. Saxena[3,4], G. Thorleifsson[5], N. Soranzo[6,7] for MAGIC* 1) Oxford, UK; 2) Cambridge, UK; 3) Cambridge, MA; 4) Boston, MA; 5) Reykjavik, Iceland; 6) Hinxton, UK; 7) London, UK.

MAGIC (Meta-Analyses of Glucose and Insulin-related traits Consortium) represents a collaborative effort to identify genetic loci associated with fasting glucose (FG), insulin (FI) and related traits. We conducted a genome-wide association meta-analysis in European descent populations from the GEM, DFS, ENGAGE and Framingham groups, combining data from 10 individual studies (deCODE, NFBC66, NTR/NESDA, Rotterdam, CoLaus, TwinsUK, DGI, FUSION, SardiNIA and FHS), comprising a total of 36,610 individuals for FG and ~26,800 individuals for FI and indices of beta-cell function (HOMA-B) and insulin sensitivity (HOMA-IR). We tested for trait associations under an additive model for a total of ~2.5 million common HapMap SNPs (directly genotyped and imputed). In the FG meta-analysis, we observed a marked excess of signals exceeding stringent significance threshold. Three of them were at the previously-reported signals at *GCK* ($p=4.8x10^{-20}$), *GCKR* ($p=1.2x10^{-8}$) and *G6PC2* ($p=7.1x10^{-49}$). We also detected a novel signal at *MTNR1B* ($p=1.1x10^{-41}$). All of these (except *GCKR*) were detected in the HOMA-B meta-analysis with the glucose-raising allele indicative of reduced beta cell function (e.g. *MTNR1B*, $p=1.0x10^{-15}$, see abstract Langenberg C. et al.) In addition to these four, there were 8 additional independent signals for FG ($p<10^{-5}$). Two of these coincided with known Type 2 Diabetes susceptibility genes (*TCF7L2* $p=2.8x10^{-11}$, *SLC30A8* $p=1.1x10^{-7}$), the strongest other signal being associated with a novel locus on chr7p ($p=3.7x10^{-12}$). These FG signals dominated the HOMA-B meta-analysis, though a slight excess of additional significant signals ($p <7.9x10^{-6}$) was observed, two of which were also detectable from the insulin and HOMA-IR meta-analyses ($p<10^{-5}$). Large-scale replication efforts currently underway should extend the list of loci with a robust effect on glycaemic traits in adults. Both physiological (homeostatic) and pathological (diabetes-related) mechanisms seem to be implicated in the regulation of these traits.

Fibulin polymorphisms and subclinical atherosclerosis, blood pressure and arterial stiffness: The Multi-Ethnic Study of Atherosclerosis (MESA). *J. D. Vargas[1], K. Musunuru[1], M. F. Cotch[2], S. D. Adar[3], D. A. Bluemke[1], L. J. Raffel[4], X. Guo[4], B. Fang[4], W. S. Post[1]* 1) Jonhs Hopkins Hospital, Baltimore, MD; 2) National Eye Institute, NIH, Bethesda, MD; 3) University of Washington, Seattle, WA; 4) Cedars-Sinai Medical Center, Los Angeles, CA.

The fibulins, a family of proteins involved in the development of the extracellular matrix, are determinants of normal arterial morphology. Single nucleotide polymorphisms (SNPs) in fibulin-3 (EFEMP1), fibulin-5 (FBLN5), fibulin-6 (HMCN1) and fibulin-3 associated protein, matrix metalloproteinase-3 (MMP-3), may play a role in the development of atherosclerosis and blood pressure homeostasis. 2,847 participants without known cardiovascular disease in the Multi-Ethnic Study of Atherosclerosis (MESA) from 4 racial/ethnic groups [African-American (AFA), Chinese (CHN), White (EUA), and Hispanic (HIS)] were genotyped. Single SNP analyses were performed to determine the associations between genotypes and arterial phenotypes, including coronary artery calcium (CAC), carotid intima-media thickness (IMT), carotid artery distensibility (CD), systolic blood pressure (SBP), diastolic blood pressure (DBP) and pulse pressure (PP). Multivariate regression analyses were used adjusting for age and gender, stratified by racial/ethnic group. An additive model was used to analyze SNP-phenotype associations with a p-value of < 0.05 in two ethnic groups indicating significance. 95 SNPs were genotyped [FBLN5 (32), EFEMP1 (25), HMCN1 (27), MMP-3 (11)]. FBLN5 rs929608 was associated with IMT in AFA and EUA (p=0.03 and p=0.01), rs7143288 with IMT in HIS and EUA (both p=0.02) and rs6575221 with CD in CHN and EUA (both p=0.03). EFEMP1 rs7589466 was associated with CAC in HIS and EUA (p=0.01 and p=0.001), rs727877 with IMT in HIS and EUA (p=0.04 and p=0.02), rs727878 with CD in AFA and EUA (both p=0.03) and rs7573078 with DBP in CHN and HIS (p=0.04 and p=0.01). HMCN1 rs10127540 was associated with CAC in AFA and EUA (p=0.006 and p=0.03) and rs1407136 with CAC in AFA and EUA (p=0.002 and p=0.01); these two SNPs are in LD (r2>0.94). There were no significant associations with MMP-3. Fibulin genes may influence blood pressure and atherosclerosis.

Novel Association of HK1 with Glycated Hemoglobin in a Non-Diabetic Population: A Genome Wide Evaluation of 14,618 Participants in the Womens Genome Health Study. *G. Pare[1], D. Chasman[1], A. N. Parker[2], J. P. Miletich[2], R. Y. L. Zee[1], P. M. Ridker[1]* 1) Center for Cardiovascular Disease Prevention, Brigham and Womens Hospital, Harvard Medical School, Boston, MA; 2) Amgen, Inc., Cambridge, MA.

Type 2 diabetes is a leading cause of morbidity and mortality. While genetic variants have been found to impact on the risk of type II diabetes mellitus, relatively few studies have focused on glycated hemoglobin, a marker of the mean blood glucose concentration of the preceding 8-12 weeks formed through non-enzymatic glycation of hemoglobin. Prospective randomized clinical trials have documented the relationship between glycated hemoglobin and the development of chronic complications in diabetics, and higher glycated hemoglobin levels, even within reference intervals, have been shown to predict type 2 diabetes risk and cardiovascular disease in healthy individuals. To comprehensively address the issue of common genetic determinants of glycated hemoglobin, we report the results from a genome wide association study of glycated hemoglobin, evaluating 337,343 SNPs in 14,618 apparently healthy Caucasian women. We demonstrate that glycated hemoglobin is associated with genetic variation at the GCK (rs730497; P=5.7 x 10-12), SLC30A8 (rs13266634; P=5.4 x 10-8), G6PC2 (rs1402837; P=4.7 x 10-10), and HK1 (rs7072268 and rs2305198; 1.8 x 10-25 and 4.3 x 10-19, respectively) loci. While associations at the GCK, SLC30A8 and G6PC2 loci confirm or extend previous work done on the genetic basis of diabetes or fasting glucose, the findings at HK1 are novel. Moreover, we were able to replicate this novel association in an independent validation sample. HK1 encodes for the enzyme hexokinase, the entry point of glucose in glycolysis and a likely candidate for glucose metabolism and diabetes. This genetic observation therefore paves the way for further studies of the biology of HK1 in hemoglobin glycation, glucose metabolism and diabetes.

Modifier genes analysis in mexican cystic fibrosis patients. *C. N. Sanchez Dominguez[1,2], M. A. Reyes Lopez[1], A. E. Bustamante Saenz[2], M. C. Villalobos Torres[2], R. Ortiz Lopez[2]* 1) Centro de Biotecnologia Genomica, Instituto Politecnico Nacional. Tamaulipas, Mexico; 2) Biochemistry Department, School of Medicine, Universidad Autonoma de Nuevo Leon. Nuevo Leon, Mexico.

Introduction: Cystic fibrosis (CF) is the most common monogenic disease in Caucasian population, its frequency is about 1/2500 live born. The estimated frequency in Hispanic population is about 1/8500. The detection rate among Hispanic CFTR mutation carriers is about 52% compared to 70 to 97% in all other Caucasians. The phenotype of CF, specially in the pulmonary disease, range from very mild symptoms reaching adult age to a fulminant course resulting in death within the first year of life. Recent studies are focused on modifier genes. These are genes that could influence the CF phenotype, acting trough inflammation, repair and remodeling, protease- anti protease balance, innate immune response, among others mechanisms. This work shows results in CF diagnosis and modifier genes analysis in Mexican population. Methods: We included 76 CF patients analyzed for CFTR mutations by PCR and reverse hybridization with allele specific oligonucleotides probes or multiplex PCR. For frequency analysis of modifier genes, we tested Alpha 1 Antitrypsin (AAT) Z and S alleles, Tumor Necrosis Factor Alpha (TNF) -308, and Interleukine 8 (IL8)-251A/T polymorphisms. Results: F508 allele was present in one or both alleles in 86.8% of the CF patients. Allele frequency of F508 was 61.8% followed by 6542X, 2789 +5G/A, S549N and 3849+10kb (ranking from 5.3 to 2.6%). 21.1% of the mutant alleles remain undetected. Preliminary results in modifier genes showed low frequency for AATS mutant allele (1%) whereas Z allele was not detected. Allele frequency for TNF -308 was higher in patients than controls (8.7% vs 2.5%). For IL8, both alleles are represented in both groups, being higher the heterozygote genotype in patients (53% vs 38%). Conclusions: F508 was present in 82.5% of the CF patients, with an allele frequency of 61.8%. TNF mutant allele had higher tendency in patients, as well as heterozygote -251A/T IL8 polymorphism.

Association of Preanalytical Variation with DNA Quality in the Prostate, Lung, Colorectal and Ovarian (PLCO) Cohort. *L. E. Mechanic[1], T. Sheehy[2], D. Carrick[1], A. Varanasi[1], K. Pettit[1], A. Khan[1], M. Cosentino[2], B. O'Brien[1]* 1) Health Studies, Westat, Rockville, MD; 2) SAIC-Frederick, Inc., Frederick, MD.

The field of genetic epidemiology has witnessed a revolution due to the launch of the International HapMap project describing the linkage disequilibrium of the human genome and the advent of high density genotyping platforms. These platforms enable genotyping of millions of single nucleotide polymorphisms (SNPs) in a single individual. Despite such marked success, the majority of statistically significant SNPs identified in genome wide association or candidate gene studies fail to replicate. The quality of DNA used in genotyping assays influences genotyping success and may influence the results. We hypothesized that the quality of DNA used in genomic assays was modulated by the type of biological specimen, method of DNA extraction, and variation in collection and storage. We investigated this hypothesis using data obtained from specimens selected for a case-control study nested within the Prostate Lung Colorectal and Ovarian (PLCO) screening trial examining association of genetic variation with breast cancer. DNA quantity and quality was assessed using Nanodrop assays. Specimens with concentrations greater than 25 ng/l and total yields of at least 20 g were considered high quality DNA for genotyping based on standards established by the NCI Core Genotyping Facility (http://cgf.nci.nih.gov/). The number of high quality DNA samples for genotyping purposes and average DNA amounts per volume of starting specimen obtained after DNA extraction were compared for each method of DNA extraction and type of biological specimen. The association of DNA quality with time between blood collection and freezing and storage time was also examined. Overall, all specimen types and methods of DNA extraction yielded DNA of sufficient quality and quantity for genotyping. Determining how DNA quantity and quality are affected by preanalytic variables will enable investigators to optimize the design and implementation of genetic association and candidate gene studies.

The origin of Native Americans from a mitochondrial DNA viewpoint. *U. A. Perego[1,2], A. Achilli[2,3], L. Milani[4], M. Lari[4], M. Pala[2], A. Olivieri[2], B. Hooshiar Kashani[2], J. E. Gomez-Palmieri[1], N. Angerhofer[1], A. Pollock[1], K. H. Ritchie[1], N. M. Myres[1], S. R. Woodward[1], D. Caramelli[4], A. Torroni[2]* 1) Sorenson Molecular Genealogy Foundation, Salt Lake City, UT, USA; 2) Dip. di Genetica e Microbiologia, Università di Pavia, Pavia, Italy; 3) Dip. di Biologia Cellulare e Ambientale, Università di Perugia, Perugia, Italy; 4) Dip. di Biologia Evolutiva, Università di Firenze, Firenze, Italy.

America, the last continent to be colonized by modern humans, is characterized by an extraordinary linguistic and cultural diversity. Until recently, it was generally believed that starting around 13,500 years ago, the first Paleo-Indians arrived from Beringia, passing through an interior ice-free corridor in western North America, and spread rapidly all the way to Tierra del Fuego. Today, we realize that the peopling of the Americas involved a much more complex process. As for the maternally transmitted mitochondrial DNA (mtDNA), it has been clear since the early nineties that Native Americans could be traced back to four major maternal lineages (haplogroups) of Asian affinity. These were initially named A, B, C and D, and are now termed A2, B2, C1 and D1. More than 95% of living Native Americans belong to these four haplogroups, which can be considered pan-American, because they are shared by North, Central and South American populations. Later, five additional maternal lineages were discovered and named X2a, D2, D3, C4c, and D4h3. These less common or rare haplogroups are restricted only to some Native American populations or geographic areas and bring the overall number of Native American mtDNA lineages to nine. Our comprehensive overview of the four pan-American branches of the mtDNA tree suggests a scenario with a human entry and spread into the Americas from Beringia about 20,000 years ago, and preliminary data raise the possibility that the uncommon five Native American haplogroups might have marked additional migratory events from Asia or Beringia. Overall, through a combined analysis of modern and ancient Native American mtDNA, we are making an effort for reconstructing the complex pre-Columbian history at both macro- and micro-geographic levels.

***PTCH1* duplication in a family with microcephaly and mild developmental delay.** *K. Derwinska[1,2], M. Smyk[1,2], M. L. Cooper[1], P. Bader[3], S. W. Cheung[1], P. Stankiewicz[1,2]* 1) Dept. of Molecular & Human Genetics, Baylor College of Medicine, Houston, TX; USA; 2) Dept. of Medical Genetics, Institute of Mother and Child, Warsaw, Poland; 3) Cytogenetics, Parkview Memorial Hospital, Ft Wayne, IN, USA.

With the exception of the X chromosome, genomic deletions are more prevalent than duplications. Due to a lack of accurate diagnostic methods, submicroscopic duplications have been under-ascertained. The development of array CGH has enabled detection of chromosomal microduplications with nearly the same sensitivity as deletions, leading to the discovery of previously unrecognized syndromes. Using a clinical targeted oligonucleotide array (CMA-V6.3 OLIGO), we identified an ~360 kb duplication in 9q22.32 in a 21-month-old boy with developmental delay, failure to thrive, and microcephaly. The same duplication was identified in the patients mother who has also microcephaly. We have sequenced the chromosomal breakpoints and determined the duplication as tandem in orientation and 363,599 bp in size. The proximal duplication breakpoint mapped within a unique sequence, 229 bp from a 6421 bp LINE1 retrotransposon element L1PA7 and the distal breakpoint mapped within a 492 bp DNA/MER1_type element Charlie2a. The duplicated segment harbors only the entire *PTCH1* gene and a non-coding exon 1 of the *FANCC* gene. To date, over 160 mutations (loss-of-function, mostly truncating) and more than 20 constitutional deletions of entire *PTCH1* have been reported in patients with Nevoid Basal Cell Carcinoma syndrome, or Gorlin syndrome. In contrast, gain-of-function mutations in *PTCH1* lead to holoprosencephaly 7 (HPE7). *PTCH1* encodes a receptor for SHH and suppresses the SHH signaling pathway. Mutations in *SHH* are the most common identified cause of both autosomal dominant and sporadic HPE; however, they have been found also in patients with microcephaly without holoprosencephaly. Furthermore, up to 50% of children with HPE have been found microcephalic at birth. We suggest that gain-of-function mutations or duplication of *PTCH1* may result in microcephaly with or without holoprosencephaly. We propose that patients with microcephaly or holoprosencephaly of unknown origin should also be screen for *PTCH1* duplication.

Investigation of the role of the PRKAR1A gene and protein kinase A enzymatic activity in endometrial tumors.

E. I. BIMPAKI[1], A. M. TSIGGINOU[2], S. A. BOIKOS[1], M. NESTEROVA[1], C. PAPAGEORGIOU[2], C. A. STRATAKIS[1] 1) Section on Endocrinology and Genetics, Program on Developmental Endocrinology & Genetics (PDEGEN), NIH, Bethesda, MD; 2) 1st Obstetrical and Gynecological clinic, 'Alexandra' Hospital, Athens, Greece.

The PRKAR1A gene is coding for the type 1a regulatory subunit of the cAMP-dependent protein kinase A (PKA), an enzyme with an important role in cell cycle regulation and proliferation. Alterations in RIA protein levels were detected in various tumors and PRKAR1A inactivating mutations lead to Carney complex (CNC), a multiple endocrine neoplasia syndrome. In this study, we investigated the role of PRKAR1A in endometrium tumors. Specimens were collected from 31 patients with endometrial cancer. In 26 of these patients, a sample from the surrounding normal tissues was also collected; in addition, 15 samples of endometrium were collected from normal controls that underwent gynecological operations for other reasons. In all samples, we 1) sequenced the PRKAR1A coding sequence; 2) determined the PKA activity and cAMP binding capacity; and 3) studied the PKA subunit expression by protein studies (immunoblotting, immunohistochemistry). There were no PRKAR1A mutations. In 5 controls and in 9 tumor samples we found the previously described polymorphism IVS3-5dupT (p value not statistically important); in 6 samples it could be determined that this was germline. Also, in the germline state, one synonymous polymorphism (c.87G>A/p.Ala29Ala) was found. cAMP binding was lower in tumor tissue compared to normal endometrium both within the same samples and normal samples from controls (12 samples/group were tested, p value<0.05).This finding was in agreement with the observation that free PKA activity was higher in tumor samples compared with control tissue (12 samples/group were tested, p value<0.05). Accordingly, PKA regulatory subunit protein levels,both RIA and RIIB were lower in tumor samples. We conclude that despite the apparent lack of frequent PRKAR1A mutations in endometrial cancer, there are widespread changes of the PKA system that suggest an involvement of cAMP signaling in this tissues malignant transformation.

Variation in *GIGYF2* is not associated with Parkinson disease. *W. Nichols[1,2], D. Kissell[1], N. Pankratz[3], M. Pauciulo[1], V. Elsaesser[1], K. Clark[1], C. Halter[3], A. Rudolph[4], J. Wojcieszek[3], R. Pfeiffer[5], T. Foroud[3], PSG-PROGENI Investigators* 1) Cincinnati Children's Hospital Medical Center, Cincinnati, OH; 2) University of Cincinnati School of Medicine, Cincinnati, OH; 3) Indiana University Medical Center, Indianapolis, IN; 4) University of Rochester, Rochester, NY; 5) University of Tennessee Health Science Center, Memphis, TN.

Parkinson disease (PD) affects nearly one million Americans and is the second most common neurodegenerative disorder. We have previously reported linkage to chromosome 2q36-37 in a sample of multiplex PD families, with the strongest evidence of linkage obtained using the subset of the sample having the strongest family history of disease and meeting the strictest diagnostic criteria. A recent study reported that mutations in *GIGYF2* result in PD and may account for the previously observed linkage finding. We sequenced 96 unrelated PD patients used in our original study that contributed to the chromosome 2q36-37 linkage signal and have subsequently genotyped our entire sample of 566 multiplex PD kindreds as well as 359 controls to test whether variants in *GIGYF2* are causative or increase susceptibility for PD. We detected three novel variants as well as one of the seven previously reported variants, but did not detect consistent evidence that these variants segregated with PD in the families. We also did not find a significant increase in risk for PD among those inheriting variants in *GIGYF2*. With the identification of so few potential *GIGYF2* mutations in these families, it is very unlikely that these few variants, observed in only a total of five families, could have accounted for the substantial linkage evidence (LOD=5.1) reported in our sample. Therefore, we do not believe that variation in *GIGYF2* accounts for the previously reported linkage finding on chromosome 2q36-37. We hypothesize that there is another gene within this chromosome 2q region which when mutated results in familial PD. Studies are ongoing to identify this gene(s). This project was supported by NS37167.

Assessing the contribution of genomic rearrangements to X-linked mental retardation: high resolution arrayCGH analysis of the IGOLD cohort. *F. L. Raymond[1], A. Whibley[1], P. S. Tarpey[2], V. Plagnol[1], R. Francis[1], R. Smith[2], P. A. Futreal[2], M. R. Stratton[2], i. GOLD consortium[1,2,3,4,5]* 1) Medical Genetics, University of Cambridge, Cambridge, United Kingdom; 2) Sanger Institute, Hinxton, Cambridge UK; 3) Greenwood Genetics Centre, South Carolina USA; 4) University of Adelaide, Australia; 5) Hunter Genetics, Newcastle, Australia.

We have previously described the systematic resequencing of >700 X chromosome genes in the IGOLD cohort of >200 males with mental retardation (MR) and transmission compatible with X-linked inheritance. We have identified sequence variants in several novel MR genes and attributed pathogenicity in a significant proportion of the cohort families. However, the cause of MR in the majority of these families remains unidentified. To address the contribution of genomic rearrangements, a mutational class inefficiently detected by capillary resequencing, we designed an X-chromosome-specific Nimblegen 385K format microarray to interrogate copy number variation by comparative genomic hybridization (CGH). Our array design targeted coding regions and evolutionarily conserved elements but also maintained high density coverage across non-coding regions. The IGOLD cohort previously screened by resequencing was investigated with this high resolution array. Pathogenic variants have been identified in >10% of cohort families, with aberrations ranging from 2kb to >3Mb in size. Most pathogenic deletions and duplications occur in only a single family, although duplications of MECP2 or HSD17B10 /HUWE1 were detected recurrently, albeit with unique breakpoints. Junction fragments have been obtained where possible, providing insight into the mutational mechanism underlying these genomic imbalances. The challenges of analysing and interpreting data generated using oligo-based arrayCGH are also discussed.

Enhanced activation of p97.ARSA fusion enzyme by sulfatase-modifying factor I for brain targeted therapy of Metachromatic leukodystrophy. *D. Y. Hou[1], M. A. Potter[2]* 1) Medical Sciences, McMaster University, Hamilton, Canada; 2) Pathology and Molecular Medicine, McMaster University, Hamilton, Canada.

Metachromatic leukodystrophy (MLD) is a neurodegenerative lysosomal storage disorder that arises from the deficiency of the lysosomal enzyme Arylsulfatase A (ARSA). ARSA undergoes post-translational activation via conversion of a conserved active site cysteine residue into a C-formylglycine residue. This modification is executed by sulfatase-modifying factor I (SUMF1). Severe deficiency of ARSA leads to lysosomal accumulation of sulfatide, progressive demyelination and neurodegeneration. The challenge facing gene therapy for MLD is the delivery of active ARSA across the blood-brain barrier (BBB) at levels sufficient for correction of sulfatide storage. P97 is a cell-surface glycoprotein for which BBB transcytosis has previously been demonstrated. To circumvent the BBB for MLD therapy, we have developed an expression vector fusing p97 and human ARSA to deliver enzyme across the BBB. MDCK cells were transfected with unmodified ARSA and p97.ARSA. We investigated whether co-transfection of MDCK cells with expression vectors for SUMF1 and either p97.ARSA fusion enzyme or unmodified ARSA resulted in an increase in secretion of active enzyme compared to cells without added SUMF1. Transfected MDCK cells expressed and secreted intact fusion proteins as confirmed by immunoblot using antibodies against p97 and ARSA. Enzyme activity assays with artificial substrate P-Nitrocatechol sulfate demonstrated that ARSA activity was not impaired after p97 fusion. The maximal secretion of unmodified ARSA and p97.ARSA from transfected MDCK cells were 290 and 600 nmoles/hr/10^6 cells respectively. Following SUMF1 co-transfection, secretion of activated unmodified ARSA was increased 7-fold to 2130 nmoles/hr/10^6 cells. Secretion of activated p97.ARSA increased 4-fold to a maximum of 2245 nmoles/hr/10^6 cells. The similar maximal secretion of active enzyme for both ARSA and p97.ARSA transfected cells after SUMF1 co-transfection suggests complete activation of produced enzyme. These results demonstrate the importance of SUMF1 in enhancing activation of p97.ARSA for brain targeted gene therapy for MLD.

Microdeletion at 9q33.3q34.11 encompassing the LMX1B and ENG genes: a case report. *P. Trapane[2], J. Jarzembowski[2,1], D. Bick[2], W. Rhead[2], D. Wargowski[4], S. Aradhya[3], A. Mitchell[3], R. Veith[1]* 1) Children's Hospital of Wisconsin; 2) Medical College of Wisconsin; 3) GeneDx; 4) University of Wisconsin School of Medicine.

We report a male neonate with multiple congenital anomalies including coarctation of the aorta, tortuous aorta, hypoplastic left ventricle, mild aortic valve stenosis, cleft palate, alveolar ridge cleft, hypospadias, midline sternal skin tag, widening of the cranial vault sutures, hypertelorism, low set ears, epicanthic folds, short and broad nose, cerebral arteriovenous malformation, broad hands, short first and fifth metacarpals, underdeveloped middle phalanx of the 5th rays bilaterally, hypoplasia of nails bilaterally, absent patellae, hindfoot deformity and widely spaced first and second digits on the feet. Prior to death at one month of age the patient developed cholestatic jaundice and hapatomegaly. No other individuals within this pedigree demonstrate the phenotype.

Utilizing oligonucleotide array CGH we identified a genomic microdeletion at 9q33.3q34.11. The deleted segment is approximately 3.7 Mb and involves a contiguous region of single copy loss of over 40 genes including LMX1B and ENG. Quantitative PCR analysis of the CRAT gene in the deleted interval confirmed a heterozygous deletion of this region. Haploinsufficiency of LMX1B causes nail-patella syndrome (OMIM 161200), a syndrome characterized by aplastic/hypoplastic nails, small or absent patellae, elbow abnormalities, and the presence of iliac horns. Approximately 15% of cases involve a deletion of a part or of the entire gene. Haploinsufficiency for ENG is associated with Hereditary Hemorrhagic Telangiectasia type I (HHT type 1, OMIM 187300). HHT type 1 is an autosomal dominant, highly penetrant vascular dysplasia leading to telangiectases and arteriovenous malformations of the skin, mucosa and viscera typically affecting the brain, liver and lung tissues. Other genes in the deleted interval include AK1 and LRRC8A. The phenotype of the patient demonstrates the utility of oligonucleotide array CGH as a tool to diagnose contiguous gene deletion syndromes unable to be detected by standard cytogenetics.

Placebo-Controlled Study of Alglucosidase Alfa in Adults with Pompe Disease. *A. Skriner[1], P. R. Clemens[2], D. Corzo[1], D. Escolar[3], J. Florence[4], P. Laforet[5], S. Lake[1], J. Mayhew[3], A. Pestronk[4], R. Rosenbloom[6], M. Wasserstein[7], A. van der Ploeg[8]* 1) Genzyme Corporation, Cambridge, MA; 2) University of Pittsburgh, Pittsburgh, PA; 3) Children's National Medical Center, Washington, DC; 4) Washington University School of Medicine, St. Louis, MO; 5) Institut de Myologie, Groupe Hospitalier Pitie-Salpetriere, Paris, France; 6) Tower Hematology Oncology, Beverly Hills, CA; 7) Mount Sinai School of Medicine, New York, NY; 8) Erasmus Medical Center, Rotterdam, The Netherlands.

Background: Pompe disease (also known as acid maltase deficiency) is a rare, metabolic myopathy caused by a deficiency of acid -glucosidase (GAA). In juveniles and adults, the disease is relentlessly progressive, leading to wheelchair dependency and respiratory failure. Methods: Patients were 8 years old, ambulatory, free of invasive ventilation, and had quantifiable respiratory and lower extremity muscle weakness. Patients (randomized 2:1) received biweekly alglucosidase alfa (Myozyme) 20 mg/kg IV or placebo for 78 weeks. Distance walked in the six minute walk test (6MWT) and % predicted forced vital capacity (FVC) were co-primary endpoints. Results: 90 patients (45 male, 45 female; 93% Caucasian; age range 10-70 years) were randomized. Baseline mean 6MWT distance was 327.4128.0 meters (50.1% predicted) and mean FVC was 54.614.8% predicted, indicating considerable disease burden at baseline. By last evaluation, estimated mean absolute differences of 28.113.1 meters in 6MWT distance (p=.03) and 3.41.2% in % predicted FVC (p=.003) were observed in favor of Myozyme vs. placebo. The frequency of adverse events and infusion associated reactions were comparable between groups. Three patients in the Myozyme group experienced hypersensitivity reactions and discontinued treatment. One patient in the Myozyme group died from causes unrelated to treatment. All evaluable patients in the Myozyme group (n=59) developed IgG antibodies to rhGAA. A trend toward decreasing IgG titers was observed. Conclusions: In this first placebo-controlled study of rhGAA in juveniles and adults with Pompe disease, Myozyme was shown to improve walking and pulmonary outcomes compared to placebo.

Sex differences in repetitive stereotyped behaviours in autism: implications for genetic liability. *P. Szatmari[1], X.-Q. Liu[2], A. D. Paterson[2,3], The Autism Genome Project Consortium* 1) Department of Psychiatry and Behavioural Neurosciences, McMaster University, Hamilton, Ontario, Canada; 2) Program in Genetics and Genome Biology, The Hospital for Sick Children, Toronto, Ontario, Canada; 3) Departments of Public Health Sciences, Psychiatry and the Institute of Medical Sciences, University of Toronto, Toronto, Ontario, Canada.

Background: The specific mechanisms underlying male predominance in autism remain a mystery. One hypothesis is that the genetic liability for autism is normally distributed in the population, and males and females have different genetic thresholds. As a consequence, we would expect that male siblings of the female probands will possess a similarly high genetic load as their sisters, and they might show greater severity or more symptoms than affected males from sibships with no affected females. Methods: Families with at least two individuals diagnosed with autism spectrum disorders (ASD) were collected from ten Autism Genome Project (AGP) sites in North America and Europe. Of the 970 families selected for the analysis, there were 631 male-only families (MO), 51 female-only families (FO) and 288 female-containing families (FC). Four gender categories were used in a mixed linear model with family as a random effect: male ASDs from MO, male ASDs from FC, female ASDs from FO and female ASDs from FC. In addition, we added covariates such as AGP site, verbal/non-verbal status and the age at ADI-R completion to the model. Results: After the adjustment for covariate effects, the repetitive stereotyped behaviour (RSB) domain total scores were significantly different across the four gender groups ($p < 0.0001$). In general, the female ASDs had lower RSB scores than the male ASDs ($p < 0.0001$). The largest RSB score difference was observed between the female and male ASDs from FC ($p < 0.0001$). Besides the differences between the male and female ASDs, the male ASDs from FC had significantly higher RSB scores than the male ASDs from the MO ($p = 0.01$). Conclusion: These results suggest important phenotypic differences in RSB across gender in ASD families and provide support for a gender-specific threshold model of genetic liability in autism.

Deletions in 15q13.3 and 16p11.2: Genomic Disorders exhibiting Incomplete Penetrance and Variable Expressivity. *T. H. Shaikh, C. Haldeman-Englert, E. Geiger, B. Connor, J. Morrissette, K. Gripp, K. Jenny, L. Medne, D. McDonald-McGinn, J. Ganesh, M. Deardorff, N. B. Spinner, E. H. Zackai* The Childrens Hospital of Philadelphia, Phila., PA.

Genomic Disorders (GDs) can arise due to copy number alterations (CNAs) of genomic fragments containing dosage-sensitive gene(s). Many GDs result from non-allelic homologous recombination (NAHR) between segmental duplications (SDs). We have analyzed 500 patients with multiple congenital anomalies (MCA) using microarray-based analysis. We identified 5 patients with a 1.5 Mb deletion in 15q13.3 and 3 with a 550 Kb deletion in 16p11.2. Both of these recently discovered, recurrent CNAs, have been associated with abnormal phenotypes and appear to result from NAHR mediated by SDs. The 15q13.3 deletion has been associated with mental retardation and seizure disorder, whereas the 16p11.2 deletion was found to be associated with autism. Of the four 15q13.3 deletions for which parental analysis was possible, 2 were found to be de novo and 2 were inherited from a phenotypically normal mother. The observed phenotypes in our patients included developmental delay, facial dysmorphia, palatal defects, cardiac defects and obesity. None of our patients have seizures or abnormal EEGs reported previously. Of the two 16p11.2 deletions for which parental analysis was possible both were found to be de novo. The observed phenotype in our patients includes developmental and language delays, seizures, facial dysmorphia, palatal defects and cardiac defects. The more severely affected patient has hemifacial microsomia and autistic-like behavior. Current data suggests that both the 15q13.3 and 16p11.2 microdeletions may exhibit incomplete penetrance and/or variable expressivity. Our ongoing analysis includes testing for recessive mutations on the non-deleted allele of genes, other modifying gene mutations or CNAs and imprinting, especially in the 15q13.3 region. We are beginning to address these questions by collecting and characterizing more patients with these CNAs. Further elucidation of genotype-phenotype correlations in these GDs will have significant implications for clinical diagnostics and genetic counseling.

Premature NRXN1 truncations due to intragenic genomic rearrangements in two families with autism, Asperger syndrome, anxiety, depression, ADHD, developmental delay, and speech delay. *B. Wisniowiecka-Kowalnik[1,2], M. Nesteruk[1,2], S. U. Peters[1], Z. Xia[1], M. L. Cooper[1], S. Savage[3], R. S. Amato[3], P. Bader[4], S. W. Cheung[1], P. Stankiewicz[1]*
1) Dept. of Molecular & Human Genetics, Baylor College of Medicine, Houston, TX; 2) Dept. of Medical Genetics, Institute of Mother and Child, Warsaw, Poland; 3) Eastern Maine Medical Center, Bangor, ME; 4) Cytogenetics, Parkview Memorial Hospital, Ft Wayne, IN.

In addition to copy-number-variation, mutations in syndromic and non-syndromic genes have been found in patients with autism or autistic spectrum disorder (ASD). Several studies reported mutations in genes encoding neurexins in a small percentage of ASD patients. These proteins are transmembrane molecules which are essential for efficient neurotransmission. and isoforms of *NRXN1* are highly expressed in the brain and have been shown to be associated with autism, ASD, schizophrenia, cognitive and behavioral abnormalities, and alcohol and nicotine dependence. We present two families, in whom we identified an intragenic deletion and a duplication within *NRXN1* using a clinical targeted oligonucleotide array-CGH V6.5 (Agilent, 44K). Whole-genome array CGH analysis with 385,000 oligonucleotide probes (NimbleGen) narrowed the breakpoints and allowed their sequencing by long range PCR. The 378,996 bp deletion was found in a pregnant woman with Asperger syndrome, anxiety and depression and three out of her four children with autism, anxiety, developmental delay, ADHD, and speech delay. The 184,260 bp tandem duplication was found in a patient with a diagnosis of autism (by ADOS and ADI-R), speech/language delays, and cognitive delays. The evaluation of parents is pending. In both cases, we found LINE-1 retrotransposon element L1PA at one of the breakpoints, suggesting its causative role. The deletion removed exons 2-4 (isoform) and the duplication copied exons 3-5 (isoform), resulting in premature truncation of NRXN1. Our data confirm previous observations that *NRXN1* may be pathogenic in a wide variety of psychiatric diseases, including autism, Asperger syndrome, anxiety, depression, ADHD, developmental delay, and speech delay.

A novel CYBB mutation causing X-linked chronic granulomatous disease in a family with primary biliary cirrhosis and granulomatous colitis. *A. Psychogios[1], A. Maddalena[2], D. Pardi[3], NM. Lindor[1], KD. Lindor[3], KN. Lazaridis[3]* 1) Medical Genetics, Mayo Clinic, Rochester, MN, USA; 2) GeneDx, USA; 3) Gastroenterology and Hepatology, Mayo Clinic, Rochester, MN, USA.

Primary biliary cirrhosis (PBC) is an idiopathic cholestatic liver disease typically affecting middle-aged women. PBC causes florid intrahepatic bile duct lesions and loss of bile ducts resulting in cirrhosis and liver failure. Patients are often asymptomatic and 90 % test positive for serum antimitochondrial antibodies (AMA). Chronic granulomatous disease (CGD) is a rare immunodeficiency disorder caused by neutrophil bactericidal defect of the NADPH oxidase enzyme complex. 70% of patients have X-linked recessive CGD caused by mutation of the p91-phox-beta polypeptide gene (CYBB). We report a Caucasian family with PBC, CGD and granulomatous colitis. The proband is a 23-year-old female with a family history of two maternal great-uncles who died in infancy of CGD. She was presented with Crohns disease and CGD retinopathy. She had functional absence of NADPH oxidase, positive AMA and a novel heterozygous deletion of 16 nucleotides in exon 5 of the CYBB gene. This mutation, c.360_375del16 (p.Phe121ValfsX2), causes a frameshift starting with codon 121 changing this amino acid to a Val residue, creating a premature Stop codon at position 2 of the new reading frame F121VfsX2, and is predicted to result in nonsense-mediated mRNA decay or premature protein truncation. The patients mother has psoriasis, decreased NADPH oxidase, negative AMA and is heterozygous carrier of the CYBB deletion. A maternal aunt has history of resected granulomatous colitis, positive AMA, liver biopsy diagnostic for PBC and is heterozygous carrier of the CYBB deletion. Conclusion: We report for the first time a clinical association of a novel disease-associated CYBB mutation with CGD, granulomatous colitis and PBC. We hypothesize that the three granulomatous disorders may represent a spectrum of a common X-linked disorder related to deficient NADPH oxidase complex. Further research is needed to clarify the pathogenetic role of CYBB mutations in females with PBC and granulomatous colitis.

The use of genome-wide eQTL associations to identify novel genetic pathways involved in complex traits. *J. L. Min[1], J. M. Taylor[1], J. B. Richards[2], T. Watts[3], J. Broxholme[1], F. Pettersson[1], K. R. Ahmadi[2], I. Ragoussis[3], A. P. Morris[1], T. D. Spector[2], L. R. Cardon[4], K. T. Zondervan[1]* 1) Bioinformatics and Statistical Genetics, Wellcome Trust Centre for Human Genetics, Oxford, United Kingdom; 2) Twin Research Unit, King's College London,London, United Kingdom; 3) Genomics Laboratory, Wellcome Trust Centre for Human Genetics, Oxford, United Kingdom; 4) Pharmacogenetics and Medical Genetics, GlaxoSmithKline, USA.

Despite recent successes of genome-wide association studies in complex traits, many associations between clinical phenotypes and genetic variants will remain difficult to uncover because of phenotypic heterogeneity. The use of downstream biological phenotypes may provide a more powerful approach. Gene expression levels are highly variable and heritable, and are known to be strongly associated with genetic variants. This study investigates the association between 44 quantitative metabolic phenotypes and 19,828 gene expression levels in 299 twins, followed up by targeted SNP association analysis in a replication set of 2,277 female twins. Expression profiling was conducted in lymphoblastoid cell lines from 154 female twin pairs from the St. Thomas UK adult twin registry, and 57 unrelated CEU HapMap individuals, using the Illumina Sentrix Human-6 version 2 BeadChip. We found 956 probes correlating with one or multiple traits. Genome wide association analysis between 900,651 non-redundant SNPs and these probes in HapMap individuals identified 5 probes with association signals in cis and 314 probes in trans. Replication of these signals in other eQTL studies was obtained for 20% of cis signals and 8% of the trans signals. We tested 1050 SNPs associated with any of the 319 probes for a SNP-clinical phenotype association in 2,277 twins and we are currently following up significant associations. This study suggests that SNPs associated with expression levels in trans may be more clinically relevant than those in cis.

Identifying genotype-phenotype signatures in genome-wide data sets using market-basket-analysis. *T. G. Schulze[1], L. Kassem[1], E.-H. Han[2], F. J. McMahon[1], G. Karypis[2]* 1) NIMH, Bethesda, MD; 2) U of Minnesota, Minneapolis, MN.

There is a lack of methods that can model gene-gene interactions and identify robust genotype-phenotype signatures in complex disorders. We propose to use market-basket-analysis, a standard tool in retail business. This technique can discover relationships between pairs of products purchased together and can be used to uncover cross-sells and related products. In contrast to many traditional clustering methods, this approach is not hampered by the presence of missing phenotypic data, typical for collections of complex disease phenotypes. We recently published the first genome-wide association study on bipolar disorder (Baum et al. 2008) using a replication design. Here, we study these 409 US and 675 German cases. The study is based on 47 phenotypic variables and 45 SNPs replicating across the samples and representing 39 genes. We then: (1) mine the US genotype (GT) data to find a frequent GT itemset (T, I), where T is the set of cases in the US dataset that contain the itemset denoted by I. (2) Look at the phenotypic (PT) signature of the US dataset for T and determine the PT variables that are at least present in 70% of the cases and their occurrence frequency within the set T is at least 1.3 times higher than the occurrence frequency in the entire US PT dataset. (3) Using the same itemset I of GT markers, look at the German dataset to find its set of cases that contain it. Call this set T_D. (4) Finally, we look at the PT variables of the German dataset for the set of cases in T_D and find its PT signature. We identify 16 GT combinations, the PT signatures of which show a degree of similarity of 85 to 99 % between the two samples. The phenotypic signatures are made up of combinations of a limited numbers of phenotypes, such as psychosis, suicidal behavior, psychomotor agitation and retardation, as well as dysregulation of sleep, appetite, and weight. Most of these variables are highly familial and have thus long been considered important delineators of genetic homogeneity. In a next step, we will apply this method to whole 500K and 1000K chip data. Using market-basket-analysis may prove to be a powerful fresh look at genotype-phenotype dissection.

Genome-wide association scan of psoriasis implicates genes associated with T cell differentiation. *A. M. Bowcock[1], J. Ding[2], K. Callis Duffin[3], R. P. Nair[4], P. E. Stuart[4], D. Goldgar[3], C. Helms[1], T. Tejasvi[4], J. E. Gudjonsson[4], A. Menter[5], J. J. Voorhees[4], G. G. Krueger[3], J. T. Elder[4,6], G. R. Abecasis[3], Collaborative Association Study of Psoriasis*
1) Dept Genetics, Washington Univ, St Louis, MO; 2) Dept Biostatistics, Univ Michigan, Ann Arbor, MI; 3) Dept Dermatology, Univ of Utah, Salt Lake City, UT; 4) Dept Dermatology, Univ of Michigan, Ann Arbor, MI; 5) Dept Dermatology, Baylor Univ Medical Center, Dallas, TX; 6) Ann Arbor Vetrans Affairs Hospital, Ann Arbor, MI.

Psoriasis is a chronic inflammatory skin disease affecting 2-3% of the European population. T cells in psoriasis lesions belong to the Th1 (CD4+) and Tc1 (CD8+) subsets, and may include a separate population of Th17 cells. To obtain insight into the genetic basis of psoriasis, a genome-wide association scan of 1,384 Caucasian psoriasis cases and 1,414 Caucasian controls on 451,724 SNPs was performed. Highly significant signals mapped to the MHC, with the most significant signals found in the immediate vicinity of HLA-C ($p = 8.36 \times 10^{-54}$). Seventeen signals were subjected to confirmation in an independent sample of 5,048 psoriasis cases and 5,041 controls of Northern European origin. 7 loci reached genome-wide significance and genes in several different associated regions encode proteins in the same biological pathway. Strong replicated association signals implicate the IL-23 signaling pathway in psoriasis, providing significant evidence for the first time of involvement of IL23A (combined $p = 9 \times 10^{-10}$) which encodes the p19 subunit of IL23, and replicating previously reported associations with IL23R (combined $p = 3 \times 10^{-8}$) and IL12B (combined $p = 10^{-28}$) which encodes the p40 subunit of IL23. IL23 signaling has been implicated in the development of Th17 cells and both IL23A and IL12B are upregulated in psoriatic lesions. Another association was to the vicinity of the tightly clustered IL4 and IL13 genes (combined $p = 3 \times 10^{-15}$) whose products are involved in the regulation of Th2 versus Th1 differentiation. These results indicate that genetic factors contribute to the altered balance of T-cell differentiation in psoriasis.

Patterns of diversity of NADPH Oxidase genes (*CYBB, CYBA, NCF2* and *NCF4*) in human populations. *E. Tarazona-Santos[1,2], F. Lyon-Freire[1], M. Machado[1], L. Burdette[3], R. Cheen[2,4], A. Crenshaw[3], C. Fabbri[5], L. Pinto[1], B. Sestanovich[2], M. Yeager[3], S. J. Chanock[2,4]* 1) Departamento de Biologia Geral, Universidade Federal de Minas Gerais, Brazil; 2) Section on Genomic Variation, Pediatric Oncology Branch, National Cancer Institute (NCI), National Institutes of Health (NIH), USA; 3) Intramural Research Support Program, NCI-FCRDC and Core Genotype Facility, NCI, NIH, USA; 4) Laboratory of Translational Genomics, Division of Cancer Epidemiology and Genetics, NCI, NIH, USA; 5) Dipartimento di Biologia Evoluzionistica e Sperimentale, Università di Bologna, Italy.

The phagocyte NADPH oxidase is an enzymatic complex that catalyzes the reduction of oxygen to O^{2-} and generates reactive oxygen species (ROS), a critical reaction for the microbicidal activity of phagocytes. In non-phagocyte cells, NADPH oxidase produces a lower amount of O^{2-}, which overproduction is associated with neurodegenerative disorders and cardiovascular impairment. The NADPH oxidase includes two membrane-spanning polypeptide subunits, gp91-phox and p22-phox (encoded by *CYBB* and *CYBA*) and three cytoplasmic polypeptide subunits, p40-phox, p47-phox and p67-phox (encoded by *NCF4*, *NCF1* and *NCF2*). Mutations in *CYBB*, *CYBA*, *NCF1* or *NCF2* can result in Chronic Granulomatous Disease, a primary immunodeficiency. We have resequenced 35524bp including the exons, UTRs, promoters and intronic regions of *CYBB*, *CYBA*, *NCF2* and *NCF4* in 102 ethnically diverse healthy individuals. For the four genes, diversity and the recombination parameter are higher in Africans than in non-Africans, consistently with the demographic history of human populations. Although most of the patterns of diversity are compatible with the mutation-drift equilibrium model in the four studied populations, we observed an exception for *NCF2* in Asia, with a particularly differentiated haplotype structure, a modal haplotype that is rare elsewhere, low diversity and an excess of rare segregating sites ($D_{Fu\text{-}Li} = -2.67$, $p = 0.007$). This pattern of diversity is compatible with the action of positive natural selection acting on *NCF2* in Asian populations.

Segmental duplications mediate novel chromosome rearrangements. *K. Rudd, B. Bunke, J. Keene, M. Adam, D. H. Ledbetter, C. L. Martin* Dept Human Gen, Emory Univ Sch Med, Atlanta, GA.

Segmental duplications (SDs) are substrates for unequal crossing-over, mediating gains and losses of genomic intervals between highly paralogous regions. This mechanism generates recurrent chromosome rearrangements that may be associated with a clinical phenotype or normal copy number variation. "Genomic disorders" generated by unequal crossing-over have been described previously; however, given the concentration of SDs in the human genome many more remain to be discovered. We identified five novel copy number changes (CNCs) in samples from 8 individuals ascertained in our clinical cytogenetics lab using a custom whole-genome oligonucleotide array. CNCs were confirmed by FISH and inheritance testing in parents is ongoing. We have mapped the breakpoints of CNCs in 2q11.2, 2q13, 7q11.21, 7q36.1 and 16p12.1 to flanking SDs, consistent with rearrangement via unequal crossing-over. Like previously described SD-mediated rearrangements, flanking SDs are >40 kb, >98% identical, and separated by 450 kb to 2.7 Mb. The 2q11.2 *de novo* gain is 1.6 Mb and contains 16 genes. Three out of four reciprocal gains or losses in 2q13 were paternally inherited; the inheritance of one loss remains to be determined. The 2q13 CNC is 2.1 Mb and includes 6 genes. The 450 kb 7q11.21 loss includes only one gene. Inheritance is unknown, and gain of an overlapping region has been reported as a CNC in control populations. The 2.7 Mb 7q36.1 loss includes 37 genes; inheritance is unknown. The 630 kb loss at 16p12.1 is of unknown inheritance and partially overlaps with a previously reported microdeletion; however, it is flanked by different SDs, producing a smaller genomic change encompassing 5 genes. Though the mechanism of rearrangement is the same, some SD-mediated copy number changes are pathogenic while others are tolerated as benign variants. Consideration of dosage-sensitive genes, comparison to CNCs in normal populations, and parental studies inform clinical interpretation. The collection of SD-mediated rearrangements reported in this study is relevant to other clinical labs diagnosing causative genomic changes and highlights the incredible plasticity of the human genome.

TSPY and its X-encoded Homologue Interact with Cyclin B and Exert Contrasting Functions on Cyclin Dependent Kinase 1 Activity and Cell Cycle Progression. *Y.-F. C. Lau, Y. Li* Dept Medicine, University of California, San Francisco, San Francisco, CA.

Testis-specific protein Y-encoded (TSPY) gene is the putative gene for the gonadoblastoma locus on the human Y chromosome (GBY). Gonadoblastoma develop most frequently in dysgenetic gonads of XY sex-reversed individuals and intersex patients. TSPY is abundantly expressed in tumor cells of gonadoblastoma, premalignant carcinoma-in-situ precursors and testicular seminoma, as well various somatic tumors, including hepatocellular carcinoma, melanoma and prostate cancer. TSPY and an X-homologue, TSPX, harbor a conserved domain, designated as SET/NAP domain, but differ at their carboxyl termini. Ectopic expression of TSPY accelerates cell proliferation by abbreviating the G2/M stage while over-expression of TSPX retards cells at the same stage of the cell cycle. Previous studies demonstrated that the SET oncoprotein is capable of binding to cyclin B. Using various protein interaction techniques, we demonstrated that TSPY and TSPX indeed bind competitively to cyclin B at their SET/NAP domains in vitro and in vivo. TSPY colocalizes with cyclin B1 during the cell cycle, particularly on the mitotic spindles at metaphase. TSPY enhances while TSPX represses the cyclin B1-CDK1 phosphorylation activity. The inhibitory effect of TSPX on the cyclin B1-CDK1 complex has been mapped to its carboxyl acidic domain that is absent in TSPY, suggesting that TSPX could serve a normal or tumor suppressor function(s) in modulating cell cycle progression at the G2/M stage while TSPY has acquired a specialized function(s) in germ cell proliferation and meiotic division. Epigenetic dysregulation of TSPY in incompatible germ or somatic cells could promote cell proliferation and predispose susceptible cells to tumorigenesis. The present findings strongly support TSPY to be the gene for GBY, a germ cell tumor predisposition locus on the Y chromosome.

Rapid generation of conditional and null mutant alleles in the mouse using targeted trapping. *B. C. Bjork[1], F. Schnütgen[2], H. von Melchner[2], D. R. Beier[1]* 1) Div Genetics, Brigham & Women's Hospital, Boston, MA; 2) Dept Molecular Hematology, Univ of Frankfurt Med Sch, Germany.

Our ongoing recessive ENU mutagenesis screen generates phenotypes that model human birth defects, such as clefting. The *cleft secondary palate 1 (csp1)* mutation is an excellent model of nonsyndromic clefting. We are developing strategies to rapidly validate such positionally-cloned mutations. The rapid generation of multi-purpose targeted mouse alleles is an attractive approach to take to accomplish this goal. The *csp1* mutant is a hypomorphic allele caused by a splice-site mutation in the zinc finger transcription factor, *Prdm16*. Mutant palate shelves fail to elevate likely due to extrinsic defects affecting mandible and/or tongue development. *Prdm16* expression in the palate shelves and other craniofacial structures is consistent with it playing a critical role during palatogenesis.

In order to validate our hypothesis that *Prdm16* is the causal gene in the *csp1* mutant, we developed a strategy to rapidly generate targeted trapping constructs for targeted trapping (Friedel et al., 2005). This approach employs a multi-fragment gateway site-specific recombination system, and requires only the PCR-amplification of two genomic fragments flanking the integration targeting site. From a single targeting of the *rsFlpROSAbgeo** gene trap cassette (Schnütgen et al. 2005) into a *Prdm16* intron, we generated a novel multi-purpose allele of *Prdm16* that can be analyzed as 1) a functional null, 2) a reporter of endogenous gene expression and 3) a conditional null allele that employs the FLEx recombination system (Schnütgen et al., 2003). Our analysis demonstrates that this allele recapitulates the *csp1* phenotype and accurately reports the endogenous *Prdm16* expression pattern.

By crossing these trapped mice to ubiquitous *FLPe*-expressing *FLPer* transgenic mice, we generated homozygous viable mice carrying the inverted, inactive gene trap cassette. We are currently breeding these mice to various *Cre*-expressing transgenic mice to study the tissue-specific *Prdm16* loss of function.

Genome-wide analyses of gene expression in Williams syndrome. *L. Dai[2], T. Tirosh-Wagner[1], R. Weiss[2], M. Gao[1], D. Mills[3], A. Reiss[4], U. Bellugi[5], J. Korenberg[2]* 1) Dept Medical Genetics, Cedar Sinai Medical Ctr, Los Angeles, CA; 2) The Brain Institute, University of Utah, Salt Lake City, UT; 3) Dept. of Psychology, Emory University, Atlanta, GA; 4) Dept. of Psychiatry and Behavioral Sciences, Stanford University, Palo Alto, CA; 5) Laboratory of Cognitive Neuroscience, The Salk Institute for Biological Studies, La Jolla, CA.

Williams syndrome is a neurodevelopmental disorder due to the deletion of a ~1.5 Mb region of chromosome band 7q11.23. Although the features of WS are ultimately due to the decreased copy number of the ~25 genes, the critical downstream biological pathways that alter development and adult function are unknown. WS provides a unique human model in which to combine high resolution array analyses to determine the components of these pathways. In this study, we determined the deletion in a cohort of 21 subjects with WS and asked whether genome-wide approaches querying 22,000 genes at the single exon level, were capable of sensitively measuring alterations of two fold reduction in single gene transcripts. Whole genome gene expression analysis was performed on total RNA samples from 21 WS DNAs with the common deletion and 6 normal controls. RNAs were labeled using a whole transcript sense target labeling assay after an rRNA reduction. Biotinylated target was hybridized to the Affymetrix GeneChip Exon 1.0 ST (sense target) arrays containing 6.5 million probe features. Probe level data was processed with RMA analysis (Robust Multiarray Analysis) for 22,000 RefSeq supported genes, and for exon-level results. Unexpectedly, rank products analysis (RP) revealed a 25-50% (1-2 fold) reduction in expression levels across chromosome 7 Williams region transcripts as the most significant genome-wide reduction observed between normal and affected groups. Of 22,000 gene transcripts queried, WS genes represented 10 of the top 11 transcripts with reduced expression. These data indicate that genome wide analyses can be used to establish both deleted genes and non-deleted genes whose transcription is altered, providing the opportunity to identify genetic networks that mediate the features of WS.

Posterior probability of linkage genome scan in NIMH Chinese schizophrenia sample. *V. Saviouk[1], Y. H. Huang[2], M. A. Azaro[1], A. S. Bassett[3], V. J. Vieland[2], L. M. Brzustowicz[1]* 1) Dept Genetics, Rutgers Univ, Piscataway, NJ; 2) Battelle Center for Mathematical Medicine, The Research Institute at Nationwide Childrens Hospital, Columbus, Ohio; 3) Dept of Psychiatry, Univ of Toronto, ON, Canada.

The results of a genome linkage scan for schizophrenia (SZ) in the NIMH HGI Chinese family collection were reported by Faraone et al (Am J Psychiatry 163:10) with the largest nonparametric linkage z score of 2.88 for D10S2327. We have reanalyzed the autosomal genome using the posterior probability of linkage (PPL). We reviewed the clinical information and classified as phenotypically unknown all individuals with organic mental syndromes and active substance dependence. Some 4.6% of families were excluded from the PPL analysis due to lack of informativeness after these diagnostic changes. We split the sample into two subsets: those without any family members with affective diagnoses (SZ group); and those with SZ, schizoaffective disorder (SA), and bipolar disorder (BP) (heterogeneous [HET] group). Subjects with SZ (1207), SA (14) and BP (5) were all considered affected. The SZ group (558 of 578 families) had 1160 subjects affected exclusively with schizophrenia; the HET group (20 families) had 47 affected subjects (28 with SZ). Genotypes were cleaned using PEDCHECK and multiple SIMWALK runs. Sample specific genetic maps were constructed using KELVINs mapping function; SZ and HET groups were analyzed by four-point analysis using KELVIN at 2 cM resolution, and the results from each group were combined via sequential updating. The results confirmed the linkage peak on 10q22 between D10S2327 and D10S2470 with a PPL of 29% after updating (SZ 29%, HET 2%). In addition we observed a peak of 30% over D3S1311 on 3q29 coming primarily from the HET group (SZ 2%, HET 30%). Two additional regions reached PPL of 10% each: 13q32 over D13S793 (SZ 12%, HET 2%) and 19q13 between D19S178 and D19S246 (SZ 9%, HET 2%). Our results demonstrated that a conservative approach to diagnostic classification and sample stratification based on observed psychopathology in families, thorough error checking of genotypes, ethnic specific genetic maps, and use of the PPL can yield novel linkage findings for complex phenotypes.

Encephalopathic ApoCII Deficiency- A Case Report. *M. Niell, M. A. Abbott* Clinical Genetics, Baystate Medical Center, Springfield, MA.

A now 6 year old girl was noted to have severe hyperlipidemia at 1.5 months of age after her eye color changed from blue to an orange/pink color. On ophthalmoscopy, she had evidence of lipemia retinalis (elevated retinas with sub-retinal yellowish exudates, dilated vessels and distortion of retinal anatomy). Brain CT showed hypodense lipid-type deposits mostly in the right hemisphere, but deposits were also apparent in the left. Upon initial hospital admission at 1.5 months of age, her blood was found to be pink and creamy in nature. Her initial triglyceride count was 3,736 mg/dL and rose to 24,540 mg/dL and her cholesterol rose from 608 mg/dL to 2,265 mg/dL over the course of two weeks. Abdominal ultrasound revealed hepatomegaly and fatty infiltration of the liver, and echocardiogram identified interventricular septal hypertrophy and right ventricular hypertrophy. Two exchange transfusions were successful in decreasing her triglyceride and cholesterol levels to 356 mg/dL and 950 mg/dL respectively. Nonetheless she had her first seizure at 2 months of age. She has been following a strict low-fat diet since 2 months of age and is receiving additional treatment with lovastatin. She currently has significant developmental delays, visual impairment due to bilateral retinal detachments, seizures controlled by anti-epileptics, and spastic quadriparesis. She has exhibited a non-progressive course with strong developmental gains. A brain MRI at 3.5 years of age showed extensive encephalomalacia and mild-to-moderate enlargement of the ventricles with areas of hemosiderin deposition and abnormal globes. Analysis of the *APOC2* gene revealed a previously unreported homozygous deletion, c.196-197delGC, in exon 3. This deletion results in a premature termination codon and presumably a truncated ApoCII protein lacking its C-terminus, which is necessary for functional interaction with lipoprotein lipase. Although ApoCII deficiency is typically diagnosed later in life, between the second and fifth decades, following episodes of recurring abdominal pain caused by pancreatitis, there have been reports of an early onset severe form. Ours is an additional description of this dramatic encephalopathic presentation of ApoCII deficiency.

Brain mitochondrial dysfunction may contribute to phenotypes of Angelman syndrome in the Ube3a deficient mice. *H. Su[1], W. Fan[2,3], J. Vesa[1], E. P. Coskun[2,3], J.-A. Gold[1], Y.-H. Jiang[4], A. Acab[5], J. H. Weiss[5], D. C. Wallace[2,3,6], V. E. Kimonis[1]* 1) Dept of Pediatrics, University of California, Irvine, Irvine, CA; 2) Center for Molecular and Mitochondrial Medicine and Genetics, University of California, Irvine, CA; 3) Dept of Biological Chemistry, University of California, Irvine, CA; 4) Dept of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 5) Dept of Anatomy and Neurobiology, University of California, Irvine, CA; 6) Dept of Ecology and Evolutionary Biology and Pediatrics, University of California, Irvine, CA.

Angelman syndrome (AS) is a severe neurological disorder caused by deficiency of the ubiquitin protein ligase Ube3a. It is as yet unknown if mitochondrial functions are related to phenotypes of AS. We found that the Ube3a deficiency results in mitochondria that are smaller in size. There is disturbance of mitochondrial inner membranes and reduction of synaptic vesicle density in the Ube3a deficient mice when compared to wild-type by using electron microscopy. We measured the enzyme activities of mitochondrial complexes in the brain, liver, heart and muscle by using oxidative phosphorylation (OXPHOS) enzyme assays. Consistently, enzyme activities of the brain mitochondrial complexes II+III were significantly reduced ($p<0.05$, Student t test, n=5/group) in the Ube3a deficient mice when compared to the wild-type mice, which could contribute to pathophysiology of AS. Muscle mitochondrial complex IV is elevated ($p=0.05$, Student t test, n=5/group) in the Ube3a deficient mice when compared to the wild-type mice, which may represent the Ube3 deficient mices attempt to compensate cumulative energetic deficiency of the brain by upregulating skeletal muscle mitochondrial enzyme activity to increase ATP production. The observed differences in the different tissue may be related to observed imprinting of Ube3a expression in the brain. These findings may help in understanding the pathogenesis and offers a new opportunity to develop treatment that could prevent the symptoms and phenotype of AS.

Cumulative effects of risk alleles on systemic lupus erythematosus susceptibility and subphenotypes. *K. E. Taylor[1], R. R. Graham[2], G. Hom[2], S. A. Chung[1], W. Ortmann[2], A. T. Lee[3], M. Petri[4], S. Manzi[5], P. K. Gregersen[3], T. W. Behrens[2], L. A. Criswell[1]* 1) Univ. California San Francisco Div. Rheumatology, San Francisco, CA; 2) Genentech Inc., South San Francisco, CA; 3) Feinstein Inst. for Medical Research, North Shore L.I.J. Health System, Manhasset, N.Y; 4) Johns Hopkins Univ. School of Medicine, Baltimore, MD; 5) Univ. Pittsburgh, Pittsburgh, PA.

Systemic lupus erythematosus (SLE) is a genetically complex disease with heterogeneous clinical manifestations. Recent whole-genome scans have greatly expanded the number of known SLE risk alleles, but the distribution of multiple risk alleles in cases versus controls and their relationship to SLE subphenotypes has not been studied. We analyzed 10 established SLE risk alleles from the Illumina 550K array in 4 independent Caucasian SLE case series (n=1310) and independent sets of controls (n=7859). We also studied associations with clinical manifestations for the cases, namely the 11 ACR criteria, anti-double-stranded DNA (anti-dsDNA) autoantibodies, and age of onset. The mean number of SLE risk alleles was 5.91 (SD 1.91) for cases versus 4.95 (SD 1.77) for controls, linear trend in log odds $p_{trend}=3x10^{-54}$. The odds ratio for SLE risk comparing 7 or greater (24%) versus 4 or less (36%) risk alleles was 3.3 (95% CI 2.8-4.0). The number of SLE risk alleles was most strongly associated with anti-dsDNA autoantibodies ($p_{trend}=3x10^{-6}$), earlier age of onset ($p_{corr} = 0.0003$), and inversely with oral ulcers ($p_{trend} = 2x10^{-5}$), and was more significant than individual SNPs in multivariate regressions for these subphenotypes. Similar results omitting HLA-DR3 and HLA-DR2 tagging SNPs ($p_{trend}=0.0004$, $p_{corr} = 0.0003$, and $p_{trend} = 10^{-5}$, respectively) indicate that these are not due simply to the HLA. In contrast, a borderline association with renal disease (p=0.03) was more strongly associated with HLA-DR3 alone, p=0.004. We conclude that SLE risk increases approximately linearly with the number of risk alleles, which is also strongly associated with the specific subphenotypes of increased anti-dsDNA autoantibodies, earlier age of onset, and decreased risk of oral ulcers.

TUBA1A mutations are a rare cause of posterior predominant lissencephaly that resembles the LIS1-associated pattern. *R. A. Kumar[1], R. J. Harvey[3], T. D. Babatz[1], W. B. Dobyns[1, 2]* 1) Dept Human Genetics, Univ Chicago, Chicago, IL; 2) Depts Pediatrics and Neurology, Univ Chicago, IL; 3) Dept Pharmacology, The School of Pharmacy, London, UK.

Lissencephaly (LIS) is a severe cortical malformation characterized by abnormally broad gyri and a thick cortex, and by histological evidence of deficient neuronal migration. It results in severe mental retardation and epilepsy, and typically shortened postnatal survival. Our past studies have detected mutations of *LIS1* or *DCX* in 85% of children with the common classic type of LIS, leaving only 15% unexplained. Recently, mutations in the tubulin alpha 1A gene (*TUBA1A*) were associated with LIS that is more severe in posterior than anterior brain regions, resembling the LIS1 pattern. We have used direct DNA sequencing to screen for *TUBA1A* coding mutations in 66 individuals with posterior predominant LIS who were negative for mutations in *DCX* and *LIS1*. We identified two missense mutations in exon 4 in 4/66 patients (6%), which suggests that *TUBA1A* mutations will account for ~1% of classic LIS (0.06 x 15% = 0.9%). The first mutation, p.R402C, was reported previously in a 35-week gestation fetus with severe grade 1 LIS and identified here in three unrelated patients with grade 2 or 3 posterior predominant LIS. The apparently greater severity in the fetus may be accounted for by developmental considerations. The second mutation, p.G143W, is novel and was found in a child with LIS who had more severe hypogenesis of the corpus callosum and cerebellar vermis than found in most children with LIS1 mutations. We used structural modeling to predict the functional impact of p.G143W on protein function. The p.G143W substitution results in a tryptophan side chain that projects into the GTP binding site, resulting in several predicted clashes and/or contacts between atoms, thereby perturbing GTP binding. G143W is the first report of a human mutation affecting the TUBA1A GTP-binding site. In conclusion, our results indicate that *TUBA1A* mutations account for ~1% of posterior-anterior LIS. Functional analysis of G143W is underway to assess whether this mutation disrupts incorporation of TUBA1A into microtubules.

Eight new cases plus review of literature supports U-type exchange as the most frequent mechanism for inverted duplication with terminal deletion rearrangements involving most chromosomal arms. *L. R. Rowe[1], L. Rector[1], J. C. Carey[2], D. Viskochil[2], A. F. Rope[2], A. R. Brothman[1,2], S. T. South[1,2]* 1) Institute for Clinical and Experimental Pathology, ARUP Laboratories, Salt Lake City, UT; 2) Division of Medical Genetics, Department of Pediatrics, University of Utah, Salt Lake City, UT.

Chromosomal rearrangements resulting in an inverted duplication with concomitant terminal deletion were first described for the short arm of chromosome 8 in 1976. Since then, this type of alteration has been identified and characterized for most chromosome arms. Three mechanisms are commonly proposed to explain the origin of this type of rearrangement. All 3 mechanisms involve formation of a dicentric chromosome that then breaks in a subsequent meiotic division to produce a monocentric duplicated and deleted chromosome. However, the events leading to the formation of the dicentric chromosome differ between the mechanisms. In one mechanism, either parent carries a paracentric inversion. This results in formation of a loop during meiotic pairing with a recombination event occurring in the loop. In the second mechanism, inverted low-copy repeats in the same chromosome arm allow partial folding of one homolog onto itself with a recombination event between the inverted repeats. The third mechanism involves a pre-meiotic double-strand break with subsequent fusion, or U-type exchange, between the sister chromatids. The first two mechanisms require a single-copy region to exist between the duplicated and deleted regions, therefore high-resolution analysis of the rearrangement can be used to distinguish between these mechanisms. Using G-banded chromosome analysis, FISH and array CGH, we describe 8 new cases of inverted duplication with terminal deletion of 2q, 8p, 9p, 10q, 15q, 18p, 18q, and 22q. These new cases combined with previously described cases demonstrate that U-type exchange is the most frequent mechanism for this type of rearrangement involving most chromosome arms.

High-density linkage screen identifies potential dementia loci in the Amish. *A. C. Davis[1], J. L. Haines[1], L. Jiang[1], P. J. Gallins[2], N. Schnetz-Boutaud[1], L. L. McFarland[1], D. Fuzzle[1], C. Knebusch[1], M. Creason[2], L. Caywood[2], C. E. Jackson[3], W. K. Scott[2], M. A. Pericak-Vance[2], J. L. McCauley[2]* 1) Center for Human Genetics Research, Vanderbilt University Medical Center, Nashville, TN; 2) Miami Institute for Human Genetics, University of Miami Miller School of Medicine, Miami, FL; 3) Scott & White, Temple, TX.

While APOE has replicated numerous times as an Alzheimer Disease (AD) susceptibility gene, it explains less than half of the genetic risk for AD, leaving more than half of the risk unsolved. Genetic heterogeneity complicates the identification of the remaining genetic risk factors. However, using a genetically isolated, and therefore relatively homogenous, population can minimize heterogeneity. We have assessed over 1700 (132 with AD) individuals from the genetically isolated Amish populations in Ohio and Indiana. Using the Anabaptist Genealogy Database (AGDB) and its query software PedHunter, we determined kinship coefficients and the family structure of our sample. We used the GREFFA program to cluster individuals, based on kinship scores 0.0156 (second-cousins), to create sub-pedigrees from our complex multi-generational extended Amish pedigree (n>4,300 over 11 generations). We performed a genome-wide SNP linkage screen (Illumina Linkage Panel IVb) using 672 Amish individuals (103 with AD) in 21 sub-pedigrees. Suggestive linkage to AD was found for 18 SNPs across 16 independent loci (2-pt lod scores >2.0: 1q, 2p, 2q, 3q22, 3q26, 4q, 5q, 7q, 8p, 13q, 14q23, 14q24, 16p, 18q, 20q, 21q). The MQLS test of linkage and association also identified 284 SNPs with nominally significant p-values (p0.05). Subsequently, we utilized the Simwalk program to perform multipoint linkage analysis for multiple 3-SNP sliding windows across each of the regions identified by the 2-pt analysis. Five of these regions continued to demonstrate strong evidence for linkage (mpt lod >1.8: 1q, 3q22, 5q, 18q, and 21q), with three of these also demonstrating MQLS p-values 0.05 (1q, 3q22, 18q). We are currently genotyping 100 SNPs across these regions in an extended set of Amish samples to confirm and refine these results.

Association analysis of SNPs located in regions linked to enuresis in children with ADHD and enuresis. *F. Lantieri[1], T. Takeda[1], R. Deberardinis[2], J. Accardo[1], P. J. Ambrosini[3], N. J. Blum[1,2], L. W. Brown[1,2], W. Berrettini[2], J. Glessner[1], C. Kim[1], M. Devoto[1,2,4], H. Hakonarson[1,2], J. Elia[1,2]* 1) The Childrens Hospital of Philadelphia, Philadelphia, PA; 2) University of Pennsylvania, Philadelphia, PA; 3) Drexel University, Philadelphia, PA; 4) University of Rome La Sapienza, Roma, Italy.

ADHD and enuresis co-occur at higher rates than expected. Genetic factors are thought to play an important role in both nocturnal enuresis and ADHD. Several studies have reported linkage of enuresis to four chromosomal regions including 8q, 12q, 13q and 22q. We carried out a family-based association study on 51 ADHD children that were also affected by enuresis and their parents for SNPs located in these loci to address their potential role in the co-morbidity of enuresis and ADHD. TDT analysis was applied to a total of 24,945 SNPs located on chromosome 13 (3179 SNPs), 12 (6602), 8 (12817), and 22 (2347) in the 51 family trios. Several of these regional SNPs showed p-value below 0.01. However, no SNP remained significant after correction for the number of test performed. Among SNPs with p0.001 the majority clustered to specific regions less than 1 Mb in size. Moreover, none of these SNPs showed a TDT p-value lower than 0.01 in a set of ADHD trios not presenting enuresis that was used as a control sample. Thus, we cannot exclude that these loci may contribute to both enuresis and ADHD, or be involved in enuresis or ADHD independently. Since ADHD had been reported to coexist in 30-40% of enuretic children, and since these children are more likely to have the inattentive symptoms of ADHD, we are currently analyzing this subset of ADHD cases only, regardless of the enuresis trait, to determine if these loci may be related to a deficits in arousal and therefore be associated to both enuresis and the inattentive symptoms of ADHD.

Evidence for current co-evolution of fertilization genes in humans. *R. V. Rohlfs*[1], *W. J. Swanson*[1], *B. S. Weir*[1,2] 1) Department of Genome Sciences, University of Washington, Seattle, WA; 2) Department of Biostatistics, University of Washington, Seattle, WA.

Coevolving genes must undergo complementary mutations to maintain the fitness of their interaction. If a pair of coevolving genes have multiple functionally distinct alleles, matching alleles will be selected across loci. Selection for allele matching may be detectable as allelic correlation between physically unlinked loci. This sort of selection has generally been dismissed as biologically improbable due to the extremely intense selective pressure required.

Using the test statistic for composite linkage disequilibrium (CLD), we have found evidence for allelic association between genes mediating sperm-egg binding in humans. We tested candidate genes for association using 1408 members of the 1958 Birth Cohort typed with Affy 500K SNP chips. Genes proposed to encode receptors in sperm cell membrane and egg zona pellucida which allow sperm-egg recognition, ZP3 and ZP3R, show an unusual degree of correlation using a standard CLD test. We are in the process of refining a novel test for general genotypic association to confirm or refute this finding.

Our results support the hypothesis of allele matching selection, even across physically unlinked loci. It is not surprising that this sort of selection is found in fertilization since this process is known to be subject to intense selection.

Acknowledgments

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Functional dissection of ARMS2/LOC387715 protein, associated with susceptibility to age-related macular degeneration. *A. Kanda[1,2], A. Estrada-Cuzcano[2], A. Swaroop[1,2,3]* 1) Neurobiology-Neurodegeneration, National Eye Institute, Bethesda, MD; 2) The Departments of Ophthalmology and Visual Sciences , University of Michigan, Ann Arbor, MI; 3) The Departments of Human Genetics, University of Michigan, Ann Arbor, MI.

We previously reported that SNP rs10490924 at 10q26 within the ARMS2/LOC387715 gene is strongly associated with susceptibility to age-related macular degeneration (AMD) and that ARMS2 encodes a 12 kDa protein which localizes to or associates with mitochondrial outer membrane in transfected cells (Kanda et al. 2007 PNAS). These results have been validated recently (Fritsche et al 2880 Nature Genetics). To further dissect its function, we generated multiple missense and deletion mutations in the ARMS2 protein by site-directed mutagenesis. Our data demonstrate that C-terminal 10 amino acids are required for mitochondrial localization/association of the ARMS2 protein. We also performed yeast two-hybrid screening using human retina cDNA library to identify ARMS2-interactive proteins; the analysis of interacting proteins is in progress. We propose that C-terminal region of ARMS2 and its mitochondrial localization/association are necessary for physiological function.

Underprovision of guideline-recommended advice regarding genetic risk assessment for hereditary breast and ovarian cancer among U.S. women. *D. E. Levy[1,2], A. E. Shields[1,2], J. E. Garber[2,3]* 1) Center on Genomics, Vulnerable Populations, and Health Disparities; Massachusetts General Hospital; Boston, MA; 2) Dept of Medicine; Harvard Medical School; Boston, MA; 3) Division of Population Science; Dana-Farber Cancer Institute; Boston, MA.

Context BRCA1/2 testing is the most commonly used genetic test to predict cancer risk. Guidelines are available to help clinicians determine who will benefit most from testing. **Objective** To identify women at high risk of hereditary breast/ovarian cancer and develop national estimates of the proportion of high-risk women who have discussed genetic testing for cancer risk with a health professional. **Methods** We identified women aged 18 and older with no personal history of breast or ovarian cancer participating in the 2000 and 2005 National Health Interview Survey Cancer Control Supplements (n=35,116). Respondents were labeled as being at high risk of hereditary breast/ovarian cancer using one of three national guidelines and self-reported family history of cancer. Main outcomes were self-reported awareness of genetic testing for cancer risk, discussion of genetic testing for cancer risk with a health professional, and having undergone genetic testing for breast/ovarian cancer risk. We compared these outcomes across women at high vs. average risk levels. **Results** 0.92% of women met at least one guideline criterion indicating high risk of hereditary breast/ovarian cancer. Among high-risk women, 55.5% were aware of genetic testing for cancer risk, 10.8% had discussed genetic testing for cancer risk with a health professional, and 1.5%; had actually undergone such testing. Adjusting for survey year, high-risk women were more likely than average risk women to have heard of genetic testing for cancer risk (RR, 1.3, 95% CI 1.2-1.5), to have discussed genetic testing for cancer risk with a health professional (RR 5.3, 95% CI 3.8-7.6), and to have undergone genetic testing for breast/ovarian cancer risk (RR 7.1, 95% CI 2.7-18.6). **Conclusions** We find few women at high risk for hereditary breast/ovarian cancer discuss genetic testing with a health professional. Thousands of women are not receiving clinically valuable information.

Genotoxicity assessment of two mouthwashes by means of micronuclei assay in buccal mucosa cells. *A. L. Zamora-Perez[1], M. Fuentes-Lerma[1,2], C. Guerrero-Velázquez[1], R. Brihuega-Velázquez[1], B. Gómez-Meda[3], G. Zúñiga-González[4], R. Mariaud-Schmidt[1]* 1) Instituto de Investigación en Odontología, Departamento de Clínicas Odontológicas Integrales, División de Disciplinas Clínicas del Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara; 2) Departamento de Ciencias de la Salud, División de Ciencias Biológicas e Ingenierías, Centro Universitario de los Altos, Universidad de Guadalajara; 3) Instituto de Biología Molecular en Medicina, CUCS, U de G; 4) Laboratorio de Mutagénesis, CIBO, IMSS.

Researchs in the dentistry fields has allowed the knowledge of the structure and biology of dental tissues, as well as technological development, resulted in new materials, tools and techniques which indicated the need to apply methods or models to evaluate the possible genotoxic properties of it. There are many different tests for the detection of genotoxic effects, as is the micronuclei assay (MN). Mouthwashes components may vary and some contain high levels of alcohol. This produces a burning sensation caused by the chelating action in the basal layer of the exfoliated cells and this direct stimulus to the epithelium, might causes increase in the rate of cell renewal. The aim of the present work was to assess the genotoxicity of two mouthwashes by means of the MN assay in buccal mucosa cells. Twenty healthy adults volunteered to participate in the study and were divided into two groups (10 each). Samples were taken from buccal mucosa before starting treatment and 30 days after treatment. A marked increased in MNC was seen in subjects that rinse their mouth with mouthwash with alcohol compared with those without that used mouthwash without alcohol (mean (SD) 2.6 (1.0) MNC/2000 cells vs 1.3 (0.6) MNC/2000 cells, respectively. Intragroup comparisons showed no differences in the MNC frequencies in group 1, whereas the MNC frequency in group 2 varied significantly (before: 1.0 (0.6) MNC/2000 cells; after: 2.6 (1.0) MNC/2000 cells. In the present study, the frequency of MNC from buccal mucosa increased significantly in those subjects who rinse their mouth with mouthwashes who contain alcohol compared with those without alcohol.

Accommodating Population Structure in the APL Test. *M. Schmidt, E. R. Martin, R. Chung* Ctr Human Genomics, Univ Miami, Miami, FL.

APL (Martin et al. AJHG 2003) is a powerful test for association based on general nuclear family structures, allowing for multiple affected offspring and missing parental genotypes. The APL statistic is based on the difference between the observed number of alleles in affected siblings and the expected number of alleles conditional on parental genotypes. In a single population, APL correctly computes the expected number of alleles by inferring missing parental genotypes based on siblings genotypes while properly accounting for identity-by-descent (IBD). In the presence of population stratification, however, the expected number of alleles can be biased away from the null hypothesis, resulting in excess type I error. Here we propose a modification of the APL test that accommodates population structure by incorporating a population clustering algorithm based on information from non-candidate markers. The expected value in the APL statistic is calculated conditional on parental genotypes and cluster information. Variability due to clustering is accommodated in the bootstrap procedure currently used by APL. Due to efficiency considerations the bootstrap requires an algorithm that is not too computationally intensive. Preferably the algorithm is robust to outliers and LD between markers. The methods investigated include complete linkage hierarchical clustering as implemented in PLINK and Wards clustering algorithm as implemented in AWclust. The outlier problem is addressed via the gap statistic or nearest neighbor analysis. We demonstrate validity of the modified APL incorporating population stratification algorithms using HapMap data originating from distinct populations as well as simulated data with known outliers or LD. We compare the power of the APL test using different clustering algorithms under a variety of population models and different sets of genetic markers to provide practical guidelines for applying APL in stratified populations.

A genome-wide association study of attempted suicide in the GAIN bipolar study. *V. Willour[1], P. Belmonte[1], P. Zandi[2], F. McMahon[3], J. Kelsoe[4], J. Potash[1], BiGS Consortium and Bipolar Phenome Group* 1) Dept Psychiatry, Johns Hopkins Univ, Baltimore, MD; 2) Dept Mental Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; 3) National Institute of Mental Health, Bethesda, MD; 4) Dept Psychiatry, University of California, San Diego, CA.

Family, twin, and adoption studies provide strong evidence for a heritable component to attempted and completed suicide. The heritability of suicidal behavior appears to depend in part on psychiatric disorders and, importantly, to also be partly independent of them (Brent and Mann 2005). Linkage regions related to attempted suicide in bipolar disorder (BP) have been identified on 2p12 and 6q25-26. We conducted a secondary analysis of genome-wide association data from the Genetic Association Information Network (GAIN) BP sample using attempted suicide as the phenotype. This sample consists of 1034 controls and 1001 BP cases, of whom 553 had a history of attempted suicide and 427 had no history of attempted suicide (21 were unknown). Genotyping was performed using Affymetrix Genome-Wide Human SNP Array 6.0 and 729,779 SNPs passing QC were examined for association with attempted suicide in BP. We analyzed the data using polytomous logistic regression with three outcome groups: BP suicide attempters, BP non-attempters, and controls. There were 649 loci with a difference in association for BP suicide attempters vs. BP non-attempters at $p<0.001$. One of the top findings (rs10269703; p=3.01 x10-7) was in muskelin (MKLN1), a mediator of cell spreading and cytoskeletal responses to the extracellular matrix component thrombospondin I. Muskelin is expressed in rodent neurons and may be involved in synaptogenesis. The odds ratio for association of rs10269703 in MKLN1 with BP suicide attempters vs. controls was 1.3 (p= 0.002), while association with BP non-attempters vs. controls was 0.8 (p=0.003). There was no evidence for association at this locus in BP when attempted suicide was not included in the analysis. We identified several interesting candidate genes for attempted suicide in BP, including MKLN1. These findings will need further follow-up for relevance to attempted suicide and replication in other samples.

MP-CBS: a new method for multi-platform integrated analysis of copy number variation (CNV). *Y. Senbabaoglu[1], N. Zhang[3], D. Absher[5], G. Sherlock[4], R. M. Myers[5], J. Li[2]* 1) Bioinformatics Graduate Program; 2) Dept of Human Genetics, University of Michigan, Ann Arbor, MI, USA; 3) Dept of Statistics; 4) Genetics, Stanford Univ, Palo Alto, CA, USA; 5) HudsonAlpha Institute for Biotechnology, Huntsville, AL, USA.

Recent advances in high-throughput data collection have dramatically improved our ability to study gene expression changes, DNA variants, and their impact to disease risks. In many studies, multiple technical platforms were used to interrogate the same samples; and it became necessary to pool information across platforms to derive a consensus molecular profile for each sample. A motivating example is The Cancer Genome Atlas (TCGA) project, a recent, NIH-funded initiative to characterize DNA, RNA, and epigenetic abnormalities in tumors. For studying CNVs it adopted three independent platforms: Affymetrix SNP 6.0 arrays, Illumina HumanHap 550K SNP arrays, and Agilent CGH 244K arrays. An integrated analysis is expected to maximize resolution and accuracy, yet currently there is no statistically well formulated method to address the between-platform differences in probe design, assay methods, sensitivity, and analytical complexity. An initial approach is to apply the circular binary segmentation (CBS) algorithm, which iteratively searches for DNA segments of altered signal intensity. The results from three platforms are combined after segmentation. Here we propose a new method, multi-platform CBS (MP-CBS), which extends CBS by relying on a generalization of the -square statistic used in the original CBS. MP-CBS does not require standardization of data, sums statistical evidence across platforms, and makes use of the integrated statistics during segmentation. It achieves improved spatial resolution, detection power, and provides a natural consensus. We will discuss data preprocessing issues, computation efficiency, as well as efforts to independently evaluate the performance of MP-CBS by comparing the integrated analysis of Affymetrix and Illumina SNP array data on HapMap samples with fosmid library-sequencing results on the same samples.

***AGTR2* is a genetic and therapeutic modifier of Marfan syndrome.** *J. P. Habashi[1,2], J. J. Doyle[2], D. Bedja[2], H. C. Dietz[2]* 1) Div Ped Cardiol; 2) Institute of Genetic Medicine and HHMI, Johns Hopkins Univ, Baltimore, MD.

Marfan syndrome(MFS), a disorder that includes aortic aneurysm, is caused by mutations in the fibrillin-1 gene(*FBN1*). Murine models demonstrated that excess activation of and signaling by TGF causes many of the manifestations of MFS. We have shown that antagonizing TGF signaling with TGF neutralizing antibody or the angiotensin II(AngII) type 1(AT1) receptor blocker losartan attenuates aneurysm formation in MFS mice. Signaling of AngII through AT1 increases the expression of TGF ligands, receptors and activators such as thrombospondin-1, while signaling through the AT2 receptor is thought to antagonize these events in some cell types. In theory, induction of AT2 signaling by AT1 blockade may contribute to the protective effect of losartan. This position is controversial because AT2 signaling can also induce apoptosis, leading to the proposal that the dual AT1/AT2 blockade achieved by limiting the production of AngII with angiotensin converting enzyme inhibitors(ACEi) is preferable. To address this, we disrupted the *Agtr2* gene (encoding AT2; AT2KO) in fibrillin-1 targeted mice(Fbn1+/-) and followed the progression of aortic aneurysms by echocardiography. AT2KO:Fbn1+/- mice showed a larger aortic dimension than Fbn1+/- mice at 8 weeks (p0.0008), and this difference was maintained at each time point until the mice were sacrificed at 1 year of age. Both were significantly worse than wild-type mice at all time points(p0.0001), while isolated disruption of AT2 did not induce aortic enlargement. AT2KO:Fbn1+/- mice had significantly worse aortic wall architecture as compared to Fbn1+/- alone (p0.0009). We then compared losartan to the ACEi enalapril in MFS mice using hemodynamically equivalent doses. While aortic growth during the 5 months of treatment was reduced in both the losartan (p0.00003) and enalapril (p0.001) groups when compared to placebo, the growth in the losartan group was less than that seen with enalapril (p0.0002). We conclude that AT2 signaling protectively modifies MFS, that the therapeutic effect of ACEi relates strictly to AT1 blockade, and that the selective AT1 antagonist losartan results in a superior clinical outcome.

A Haplotype Spanning the SREBF1 Gene is Associated with BMI and Varies in Frequency Worldwide. *S. D. Bailey[1], G. Pare[2], A. Montpetit[2], N. Rudzicz[3], R. Do[1], D. Gaudet[4], C. Bouchard[5], L. Perusse[6], M.-C. Vohl[6], B. Keavney[7], T. J. Hudson[2], S. Yusuf[8], S. S. Anand[8], J. C. Engert[1]* 1) Dept. of Human Genetics,McGill Univ.,Montreal,QC; 2) McGill Univ. & Genome Quebec Innovation Centre, Montreal,QC; 3) McGill Univ. Health Centre,Montreal,QC; 4) Dept. of Medicine,Univ. of Montreal,Chicoutimi,QC; 5) Pennington Biomedical Res. Centre,Baton Rouge,LA; 6) Lipid Res. Centre,Laval Univ. Hospital Res. Centre, Ste-Foy,QC; 7) Univ. of Newcastle,Newcastle,UK; 8) Dept. of Medicine,McMaster Univ.,Hamilton,ON.

Significant differences in cardiovascular disease (CVD) risk exist between populations. Therefore identifying genetic variants that display differences in allele frequencies between populations may help elucidate the etiology of these population differences, as well as identify genes that have undergone recent selection. We analyzed 1536 SNPs, from 103 CVD candidate genes, for differences in derived allele frequency (DAF) in 38 populations. One gene was the sterol regulatory element-binding transcription factor 1 (SREBF1), which plays a role in lipid metabolism. Eight SNPs encompassing SREBF1 had large and similar differences in DAF between populations. This pattern was caused by two common haplotypes that accounted for 65% of all haplotypes in non-African populations. One haplotype (HapA) was found to be most common among Europeans (freq=0.37-0.68). The second most frequent haplotype in Europeans (HapB) had the opposite allele at every SNP. Individuals homozygous for HapA were found to be significantly heavier than those homozygous for HapB in the entire INTERHEART sample (p=0.007). HapA was associated with BMI in the three largest ethnic groups examined (p0.05). The frequency of HapA is highly correlated with the mean BMI for each country (r^2=0.66,p=3.8x10-5;r^2=0.2,p=0.025 for men and women respectively). We replicated the association with BMI in Quebec family-based samples, using 2 SNPs that captured the Haplotype (p0.05). Additional associations were found with total abdominal adipose tissue (AT) and subcutaneous AT assessed by CT at the L4L5 level (p0.05). Thus, this locus may explain a portion of the between population differences in BMI and adipose tissue distribution.

SNPs into the NFE2L2 regulatory region and arsenic susceptibility. *F. Centeno[1], E. Cordova[1], C. Rangel[1], K. Carrillo[1], O. Valenzuela[2], L. M. Del Razo[2], L. Orozco[1]* 1) Investigación, Instituto Nacional de Medicina Genómica, SS; 2) Centro de Investifación y de Estudios Avanzados, IPN.

Arsenic contaminated ground water is the main source of human exposure worldwide and it represents an important health issue. Arsenic exposure has been associated with skin cancer and non-cancerous skin lesion as hyperkeratosis, hyperpigmentation and acanthosis. The Nrf2 transcription factor (nuclear factor erythroid 2-related factor 2) regulates the expression of a battery of genes encoding antioxidant and phase II detoxification enzymes. Nrf2 activation has been proposed as an important mechanism against arsenic exposure in different cellular types. Thus, the aim of this study was to determine whether the association between NFE2L2 gene variants and the occurrence of skin lesions by exposure to arsenic-contaminated water. Initially, we sequence 1000 Kb of the Nrf2 regulatory region and its first non-conding exon from 120 Mexican-Mestizo healthy donors to identify novel NFE2L2 SNPs. We found three out of six (-653G/A, -651G/A and -617C/A) previously described polymorphisms in the promoter and one (12delGCC) at the 5 untranslated region sequence. Additionally, we found a novel variation in only one subject (). We also identify a new allele comprised by only two repetitions of the CCG triplet, instead of the previously reported four or five-time triplet repeat alleles. It was found in two unrelated heterozygous subjects, The -617C/A and -653G/A SNPs were genotyped by the 5'nuclease assay (TaqMan), and the 12delCCG polymorphism by Gene Scan Analysis in 117 subjects with (cases) or without (controls) arsenic-related skin lesions. Total arsenic levels was determined in urine and drinking water. Genotypic and allelic frecuencies from the analyzed polymorphisms did not show significant differences between cases and controls. Haplotype analysis showed a higher frecuency of the CA haplotype in controls with low levels of arsenic, than in cases. Our results, suggest a protective role of the Nrf2 CA haplotype against low exposure to arsenic. Increasing the sample size will allow us to confirme this data.

Efficient Reconstruction of Whole Genomes Using Massively Parallel Shotgun Sequence Data. *Y. Li, G. R. Abecasis* Dept Biostatistics, Univ Michigan, Ann Arbor, MI.

Tremendous advances in shotgun sequencing technologies make it feasible to rapidly generate gigabases of sequence data in hundreds or thousands of individuals. These new technologies represent an important advance over conventional genotyping approaches because they allow for more comprehensive assessments not only of SNP variation but also of other polymorphisms, including many types of copy number variants. To date, these technologies have been used to sample and reconstruct whole genomes for a small number of individuals by sampling non-repetitive regions of the genome high redundancy (20-30x coverage). We show that, when many individuals are examined, it is possible to use relatively modest amounts of sequence data for each individual (2-4x coverage) to reconstruct high-quality whole genome sequences. The savings is possible because, typically, short stretches of chromosome are shared between many individuals in each sample and information can be combined across these to achieve high-quality sequence. We describe a Hidden Markov Model for rapid reconstruction of whole genome sequences from shotgun resequencing data. By simulating shotgun sequence data consisting of 30bp reads with a 0.5% error rate per base-pair, we show that, using our approach, analyzing sequence data for 400 - 1,000 individuals sequenced at depth 8 achieves comparable accuracy (< 1 error / 10,000 bp at polymorphic sites) as analysis of deep 20-30x coverage using conventional approaches that don't integrate data across individuals. To illustrate the practical utility of our approach, we also analyze publicly available sequence data on ~180 individuals examined by the 1000 Genomes Project. We expect that our method will allow for the cost-effective utilization of new sequencing technologies, either for targeted analysis of specific regions or for whole genome resequencing. We expect that both these approaches will be important for identifying causal variants and for mapping rare variants that are not adequately captured by current whole genome genotyping approaches.

First neuronally expressed gene associated with multiple sclerosis. *Y. S. Aulchenko[1], I. A. Hoppenbrouwers[2], S. V. Ramagopalan[3], L. Broer[1,2], N. Jafari[2], J. Hillert[4], J. Link[4], W. Lundström[4], E. Greiner[4], A. D. Sadovnick[5], D. Goossens[6], C. van Broeckhoven[6], J. del Favero[6], G. C. Ebers[3], B. A. Oostra[1], C. M. van Duijn[1], R. Q. Hintzen[2]* 1) Epidemiology & Biostatistics and Clinical Genetics, Erasmus MC, Rotterdam, Netherlands; 2) Department of Neurology, Erasmus MC, Rotterdam, The Netherlands; 3) Wellcome Trust Centre for Human Genetics and Department of Clinical Neurology, University of Oxford, Oxford, UK; 4) Department of Neurology, Karolinska Institute, Stockholm, Sweden; 5) Department of Medical Genetics, Division of Neurology, University of British Columbia, Vancouver, Canada; 6) Department for Molecular Genetics, Antwerp Univerity, Antwerp, Belgium.

Multiple sclerosis (MS) is a complex disease resulting from genetic and environmental factors. The genetic influence on MS susceptibility is substantial, as evidenced by the 20-fold increase in risk for siblings of MS patients. Until recently, the MHC class II locus has been the only one consistently associated with MS. Given the strong evidence for an autoimmune origin of MS, it is not surprising that the major MS genes discovered by genome wide association (GWA) analysis were these related to T-cell function (*IL2R* and *IL7R*). We here report a GWA study in 45 MS cases and 195 controls in a genetically isolated population. The study has identified a new MS susceptibility locus on chromosome 1. This locus was confirmed in a replication set of 2,679 cases and 3,125 controls derived from four independent populations. A single nucleotide polymorphism, located in this newly identified gene, was significantly and consistently associated to MS in each of the four populations (combined allelic odds ratio 1.35, 95% confidence interval from 1.23 to 1.48, $p = 2.5\ 10^{-10}$). The gene identified is neuronally expressed and is involved in axonal function and thus can be directly linked to recent data on axonal suffering in MS. This risk gene for the first time draws genetic attention to the central nervous system, the target organ in this disease.

Popular representations of genetics research: Recommendations for journalist-researcher relationships. *C. Ouellette[1], T. Bubela[2], T. Caulfield[1]* 1) Health Law Institute, University of Alberta, Edmonton, AB, Canada; 2) School of Public Health, University of Alberta, Edmonton, AB, Canada.

Human genetics research receives a lot of attention from the media and the public. The media have been criticized for hype with coverage generally falling into two categories: great promise or concern. To add to concerns, new media are now a major source of information for the public. These range from news sites, allowing for expanded coverage of scientific and social issues, to corporate advertising aimed at providing genetic testing services. Providers are concerned that media coverage is inadequate; it influences the tests and treatments requested by patients and is often misinterpreted.

Our study asks whether the media coverage evidence of genomics is as bleak as it appears. We synthesize the evidence on media portrayals of genetics and genomics. Are the media overly deterministic in representing genetic causation for phenotypes and prone to error and hype? Second, we summarize communication recommendations between researchers and journalists from journal articles, commentaries and policy reports. Do these recommendations hold for new media? Are they based on a 'deficit model' of science communication, where the public would be more supportive of genetic research if only they understood the science and issues?

Evidence shows that print media accurately reflect genetic research. However, hype may be initiated by researchers quoted by journalists; coverage is generally uncritical and framed to promote economic interests and scientific progress. The majority of errors are omissions of risk factors, timelines for benefits, conflicts of interest, funding, and the rise of commercial interests in biomedical research. Omissions may contribute to the sense of hype and may decrease public trust. In the face of these issues, the internet is both a blessing and a curse. For high quality sites, the absence of space constraints and the ability to link to sources may correct the omission problem of print media. For industry representations, there is likely a need for tighter regulation of health claims and information.

PHYLOGENY OF THE PROLYL-3 HYDROXYLASE FAMILY COMPARED TO EXPRESSION. *J. Lennington[1], D. Baldridge[1], R. Morello[1], B. Lee[1,2]* 1) Molec & Human Gen, Baylor Col Med, Houston, TX; 2) Howard Hughes Medical Institute.

The family of prolyl-3 hydroxylase related proteins is required for evolutionarily conserved post-translational modification of collagens. Mutations in two of its members cause a form of OI, and dysregulation may contribute to a broader range of connective tissue diseases. The family is made up of 5 homologues: CRTAP, No55, P3H1, P3H2, and P3H3. The P3Hs all have dioxygenase domains and hydroxylate collagen prolines in the 3-trans position of Gly-X-4Hyp. CRTAP and No55 lack the functional dioxygenase domain; however, CRTAP is required for P3H1 function in a tertiary complex with cyclophilin B. All family members share a predicted common ancestor, at which point a duplication event lead to a common CRTAP/No55 branch, and common P3H branch. CRTAP is the most highly conserved family, with average of 75.8% homology to the human ortholog in vertebrates, possibly as it is required for the function of other family members. No55 has the lowest homology among vertebrates, 63.2%, and greatest number of duplication events during evolution, suggesting a less essential role. There is a separate branch initially for a P3H1 and common P3H2/P3H3 ancestor. Homology among vertebrates for P3H1, P3H2, and P3H3 is 65.2%, 74.6%, and 66.4% respectively. P3H1 and P3H3 have many overlapping regions of expression such as cortical bone and craniofacial mesenchyme. P3H2 expression is unique and includes hypertrophic chondrocytes, skeletal muscle, glomeruli, and heart. P3H3 has the largest distance between avian and mammalian ancestors, 4.7-5.9 fold greater than P3H2 and P3H1. P3H3 may be required for mammalian specific development, such as viviparous nature or more developed medulla and urinary concentrating ability. P3H3 is expressed most strongly in early murine kidney development (E13.5), and has strong expression in the medulla and interstitium of the kidney. The mammal with lowest homology to human P3H3, the armadillo, has acute interstitial nephritis and thickened tubular basement membranes in as high as 70% of animals. Examining the phylogeny of this group integral to collagen modification may further aid in understanding each members differential function.

***AFG3L2* mutations cause autosomal dominant ataxia SCA28 and reveal an essential role of the *m*-AAA AFG3L2 homocomplex in the cerebellum.** *D. DiBella[1], F. Lazzaro[2], A. Brusco[3], G. Battaglia[1], A. Pastore[4], A. Finardi[1], V. Fracasso[1], M. Plumari[1], C. Cagnoli[3], F. Tempia[3], A. Brussino[3], C. Gellera[1], C. Mariotti[1], P. Plevani[2], S. DiDonato[1], T. Langer[5], M. Muzi-Falconi[2], F. Taroni[1]* 1) Fond IRCCS Ist Neurologico Carlo Besta, Milan, Italy; 2) Dept Biomol Sci Biotec, Univ of Milan, Italy; 3) Univ of Turin, Italy; 4) Nat.l Inst Med Res, London, UK; 5) Genetics Inst, Univ of Köln, Germany.

Autosomal dominant spinocerebellar ataxias (SCA) are a heterogeneous group of neurological disorders characterized by cerebellar dysfunction mostly due to Purkinje cell degeneration. We previously mapped SCA28 to chromosome 18p11 in a 4-generation Italian family. We have now discovered that *AFG3L2* (ATPase family gene 3-like 2) mutations cause SCA28. Along with paraplegin, which causes recessive spastic paraparesis SPG7, AFG3L2 is a component of the mitochondrial *m*-AAA complex, an evolutionarily conserved metalloprotease involved in protein quality control. We have identified 4 heterozygous *AFG3L2* missense mutations in the original kindred and in other 3/129 (~2%) unrelated SCA families. Interestingly, one index patient in this latter group was found to carry also a heterozygous SPG7 recessive mutation in the paraplegin gene. Segregation analysis of the *AFG3L2* and *SPG7* mutations in this family demonstrated a modulatory role of the *SPG7* mutation, with a full-blown phenotype in the *AFG3L2*/*SPG7* compound heterozygote and cerebellar atrophy in the *AFG3L2* heterozygotes. All the AFG3L2 mutations are located in the FtsH-like protease domain at highly conserved amino acids. Modeling on FtsH structure indicates that they may affect substrate interaction. AFG3L2 protein and transcript were found to be highly and selectively expressed in cerebellar Purkinje cells. Expression of normal and mutant AFG3L2 homocomplex in *m*-AAA-deficient yeast cells demonstrate that the mutations cause respiratory deficiency and proteolytic defect. This work identifies *AFG3L2* as a novel cause of dominant neurodegenerative disease and indicates an essential role of the AFG3L2 homocomplex in protecting the cerebellum against neurodegeneration [Italian Ministry of Health grant (ex art 56) to FT].

Investigation of Lipase Maturation Factor 1 in dyslipidemia. *D. Weissglas[1], M. Péterfy[2], M. Doolittle[2], O. Ben-Zeev[2], A. Huertas-Vazquez[1], B. Aouizerat[3], J. Kane[4], I. Cruz-Bautista[5], T. Tusie-Luna[5], C. Aguilar-Salinas[5], M.-R. Taskinen[6], P. Pajukanta[1]* 1) Dept. of Human Genetics, UCLA, Los Angeles, CA; 2) Dept. of Medicine, UCLA, Los Angeles, CA; 3) Dept. of Physiologic Nursing, UCSF, San Francisco, CA; 4) Dept. of Medicine, UCSF, San Francisco, CA; 5) INCMNSZ, Mexico City, Mexico; 6) Dept. of Medicine, University of Helsinki, Finland.

Mice carrying the combined lipase deficiency (cld) mutation exhibit hypertriglyceridemia (HTG) due to decreased activities of both lipoprotein lipase (LPL) and hepatic lipase (HL). The cld mutation that we recently identified resides in a novel gene, lipase maturation factor 1 (LMF1) (Péterfy et al. Nature Genetics 2007) that promotes maturation of nascent LPL and HL. To study the potential role of LMF1 in human dyslipidemia, we resequenced the coding regions of LMF1 in 42 Finnish and U.S. Caucasian HTG cases exhibiting combined lipase deficiency. We identified 19 DNA variants: 7 intronic, 7 synonymous, 4 nonsynonymous (ns) (G36D, M117L, R364Q, R451W) and a nonsense variant (Y439X). Of the 5 coding variants, 4 were novel, and 3 were highly conserved across species. To estimate the frequency of the coding variants we genotyped them in normolipidemic controls. The frequency of the mutations was lower or zero among the controls except for G36D. Therefore, we investigated the 4 rare mutations in the mouse cld model for functional significance. We introduced wildtype LMF1 and the mutations into an LMF1 cDNA expression construct in cld/cld cells to examine LPL specific activity. Although the ns cDNA constructs rescued the cld/cld cells, we observed a decrease in specific activity of LPL for two of these ns variants. Importantly, the Y439X cDNA construct was not able to rescue the cld/cld cells. Currently we are investigating the LMF1 gene in 38 Mexican individuals with severe HTG (TG > 1000 mg/ml), to search for shared and population-specific DNA variants. Thus far, we have identified 7 intronic, 7 synonymous and 4 ns variants, of which 7 were observed only in Mexicans. Furthermore, 30% of the Mexican HTG cases had a ns variant in LMF1. In conclusion, our data show that LMF1 variants are involved in human lipase deficiencies and dyslipidemia.

VKORC1 is associated with bone mineral density and osteoporosis risk in African-Americans from the Third National Health and Nutrition Examination Survey. *M. J. Rieder[1], C. L. Sanders[2], D. C. Crawford[3]* 1) Dept Genome Sciences, University of Washington; 2) Division of Health and Nutrition Examination Surveys, National Center for Health Statistics, Centers for Disease Control and Prevention; 3) Center for Human Genetics Research, Vanderbilt University.

Osteoporosis is a common disease in postmenopausal women defined by a low bone mineral density (BMD), with both environmental and genetic factors. We hypothesized that SNPs in the vitamin K epoxide reductase complex subunit 1 (*VKORC1*) gene are associated with BMD. VKORC1 generates vitamin K quinone, a cofactor for the gamma-carboxylation of bone-related proteins such as osteocalcin. We genotyped four *VKORC1* SNPs in 7157 individuals from the Third National Health and Nutrition Examination Survey (NHANES III). NHANES III is a nationally representative sample linked to health and lifestyle variables including BMD, which was measured using dual energy x-ray absorptiometry (DEXA) on four regions of the proximal femur. In a multivariate model stratified by race/ethnicity and sex, SNPs rs9923231 and rs9934438 were associated with decreased BMD in African-American (AA) adult females ($n=953$; $p<0.01$) and males ($n=671$; $p<0.005$) while rs8050894 was associated with decreased BMD in AA males only ($p<0.001$). In multivariate models with genetic interactions for SNP*serum calcium levels rs9923231 ($p=0.0175$) and rs9934438 ($p=0.0064$) serum calcium interactions were associated in AA females with increased BMD. All four SNP*serum calcium terms were significantly associated with decreased BMD in Mexican-American (MA) males ($n=834$; $p<0.05$). No significant associations or interaction terms were observed for European-American females or males or MA females. Osteoporosis risk was tested by defining cases as participants 2.5 SD below the reference BMD mean (at 20-29 yrs). Among AA female cases ($n=19$) and controls ($n=703$), rs9923231 and rs993438 ($OR_{adj}=0.20$; 95% CI: 0.07-0.58), and rs2359612 ($OR_{adj}=0.52$; 95% CI: 0.28-0.94) were significantly associated with decreased osteoporosis risk; rs8050894 was marginally associated ($OR_{adj}=0.27$; 95% CI:0.07-1.02). These data suggest *VKORC1* may be associated with BMD and osteoporosis in the AA population.

Integration of a novel medical genetics curriculum in the family medicine junior clerkship. *K. D. McKelvey[1], S. H. Prajapati[2], B. Bendure[4], C. Paniagua[3], R. M. Smith[5], V. A. Johnson[5]* 1) Division of Genetics and Department of Family and Preventive Medicine, University of Arkansas for Medical Sciences, Little Rock, AR; 2) Department of Family and Preventive Medicine; 3) College of Nursing; 4) College of Medicine; 5) Office of Academic Affairs and Educational Development.

In recognition of the emerging importance of genetics in clinical medicine, we developed a novel curriculum to educate junior medical students regarding unique aspects of genetic tests. Implications for medical genetics include an ever-expanding role in preventive medicine, but responsible application requires critical thought into the ethical, legal, and social issues created by molecular genetics. Our interactive online curriculum is designed to accommodate students across multiple sites over a 4 week period. It uses topic-based asynchronous discussion to stimulate critical thinking during the process. Inclusion of this project into the required curriculum of the third year family medicine clerkship serves as a crucial step in the incorporation of genetics into preventive medicine. We will present data from a total of 69 students who have completed both the pre and post knowledge acquisition tests and evaluated the course to date. The mean, standard deviation, and overall change in scores will be discussed and illustrated. The primary purpose of the evaluation is continuous program improvement. Since this is a new curriculum, information that maximizes future student learning is particularly important. An examination of pre-post scores for specific course topics indicates a need for this type of curriculum as well as opportunities to improve learning. Open responses to the query Please give your suggestions for improving the material in the course will be discussed and provide valuable insight into where the content could be improved to increase the amount of learning change.

Fragile X allele frequency comparisons among different ethnicities. *A. Cronister, S. Bhatt, Y. Wang, L. Rosenblum-Vos* Genzyme Genetics, Westborough, MA.

Introduction: Carrier screening for fragile X requires complete information for genetic counseling, yet published studies comparing premutation and intermediate allele prevalence among different ethnicities are limited. Studies in Canada and Israel found a premutation frequency of 1/259 and 1/157 women, respectively. Variation in the modal distribution of allele sizes among ethnicities has also been documented. Here, we analyze Genzyme Genetics data from over 40,000 females who had fragile X carrier testing. Allele frequencies were compared among different populations. Methods: This study included >40,000 women referred for fragile X carrier testing between 1999 and 2008. Women with a family history of fragile X, MR, DD or autism were excluded. Ethnicity was self-reported by the patient. Ethnic groups were: Caucasian (n= 22655), African American (n= 3550), Asian (n= 5106), Hispanic (n= 7804) and Jewish (n= 1214). Results: Overall, the intermediate frequency was 1/45 and the premutation frequency was 1/227. The observed premutation frequency was 1/187 in Caucasians and significantly lower in Asians than in other groups (1/567; $p = 0.0013$). Intermediate frequencies were lowest in Asians (1/104) and African Americans (1/60) and differed significantly from other groups ($p < 0.0001$ and $p=0.0025$, respectively). Allelic distribution also varied among populations. The most common size allele in all groups was 30, but Asians showed minor populations at 36 and 37. Caucasians and Hispanics had sub-populations at 20-24 repeats. The percentage of women whose smallest allele was 33-44 repeats varied among populations (Asians: 2.41%, Caucasians: 1.28%, African Americans: 0.96%, Hispanics: 0.85%, Jews: 0.31%). Conclusions: Our study suggests variation in allelic distribution and intermediate and premutation frequencies in different ethnic groups. The lower premutation carrier frequency observed in Asians but higher frequency of allele pairs in the 33-44 range, is of interest considering the prevalence of fragile X syndrome is similar to other populations. Further studies within different ethnic groups are necessary to clarify the prevalence of fragile X as well as factors that may lead to instability and risk for fragile X expansion.

In depth association analysis of positional candidate region for bipolar disorder. *P. Zhang[1], S. Zöllner[1,2], Y. Chen[1], M. Burmeister[2,3], M. McInnis[2]* 1) Department of Biostatistics, University of Michigan, Ann Arbor, MI; 2) Department of Psychiatry, University of Michigan, Ann Arbor, MI; 3) Department of Human Genetics, University of Michigan, Ann Arbor, MI.

Bipolar disorder (BP) is a highly heritable trait but identifying susceptibility genes has been challenging and no findings have been reliably confirmed. Previous meta-analysis by McQueen et al (2005) showed the strongest linkage peak with LOD=3.40 in chromosome 8q region. We use fine map association analysis to further explore the 8q region for underlying risk variants. A sample of 3,553 individuals from 737 families with bipolar disorder was collected and genotyped for 1,461 tag SNPs in a 16Mb region flanking the candidate loci identified by McQueen et al. We performed genetic association analysis using LAMP. Our preliminary analysis identified three SNPs that are significantly associated with BP after correction for multiple testing: rs7845065 (p =9.80E-06), rs2668096 (p =1.40E-05), and rs7825584 (p=7.00E-05). Two genes are located within 1 Mb of the most significant SNP rs7845065, the hyaluronan synthase 2 gene (HAS2) and the zinc fingers and homeoboxes 2 gene (ZHX2). We have genotyped an additional 1536 SNPs in the same region to get more complete coverage. Further investigation is needed to confirm these results and to identify candidate genes that play a role in the biological pathway of BP.

A novel *FGFR2* Deletion in Beare-Stevenson Syndrome. *A. Slavotinek[1], H. Crawford[2], H. Perry[3], C. Tao[3], C. Fitzgerald[1], S. Oberoi[3], K. Vargevik[3], M. Friez[2]* 1) Dept Pediatrics, U585P, Univ California, San Francisco, San Francisco, CA; 2) Greenwood Genetics Center, Greenwood, SC; 3) School of Dentistry, Univ California, San Francisco, San Francisco, CA.

Beare-Stevenson syndrome (BSS) is a rare craniosynostosis syndrome characterized by cutis gyrata, acanthosis nigricans, skin furrows and tags, anogenital anomalies and a prominent umbilical stump. We present a fraternal twin male who had cutis gyrata and acanthosis nigricans of the scalp, excess neck skin, proptosis with hypoplasia of the supraorbital ridges, hypertelorism, midface hypoplasia, narrowing of the external ear canals, skin tags at the corners of the mouth, neonatal teeth, a prominent umbilicus, broad halluces, excess palmar and plantar skin and a prominent scrotal raphé. Investigations revealed an Arnold-Chiari malformation, fusion of the superior sagittal suture and bilateral choanal stenosis. A clinical diagnosis of BSS was made shortly after birth, but sequencing of exon 11 of the *FGFR2* gene for the previously reported p.Y375C and p.S372C mutations in BSS was normal. These two mutations are likely to affect both the BEK and KGFR isoforms of FGFR2 and were hypothesized to cause disease by constitutive activation of these isoforms, although functional studies were not performed (Przylepa et al., Nat Genet 1996;13:492-494). Our patient was re-studied at age six years because of a diagnosis of rhabdomyosarcoma in his twin brother. A novel, *de novo* mutation in *FGFR2* was identified, with a deletion of 62 nucleotides starting in exon 8 (IgG IIIa domain) of the gene, c.859del62, a region in which missense mutations have been associated with Crouzon syndrome. This deletion predicts haploinsufficiency for *FGFR2*, a disease mechanism previously found in lacrimo-auriculo-dento-digital (LADD) syndrome, a condition very different from BSS. This deletion provides new information concerning the mutational spectrum of BSS, the phenotypic overlap between BSS and Crouzon syndrome, and broadens the phenotypes that arise from *FGFR2* deletions.

Genomics and doping control: The application of high-resolution expression profiling of whole blood to strategies to detect erythropoietin (EPO) abuse in athletes. *J. L. Rupert, M. E. Gallo, M. N. Fedoruk* Sch Human Kinetics, Univ British Columbia, Vancouver, BC, Canada.

BACKGROUND: Erythropoietin (EPO))is a glycoprotein hormone that stimulates the maturation of red blood cells, thereby increasing haematocrit and the oxygen carrying capacity of blood. Since the late 1980s, recombinant EPO (rhEPO) has become one of the most widely abused performance enhancing substances in endurance sports. Current anti-doping tests are based on differences in glycosylation patterns between the rhEPO and naturally occurring EPO; however, doping authorities are concerned that new variants of the drug or the use of EPO gene therapy (gene-doping) may render current testing stratagems obsolete. We are investigating transcription profiling as a potential method of detecting the abuse of rhEPO or EPO gene-doping. METHODS: Erythropoiesis was induced in mice by hypoxia (13% O2) or rhEPO (10mg/kg SC). Conditions were designed to mimic the ~10% increase in haematocrit targeted by doping athletes. For each condition (and controls), blood was taken from 10 mice and pooled. LongSAGE libraries generated and sequenced using Illumina 1G technology by the Genome Science Center in Vancouver. The libraries were compared and transcripts significantly over-represented in the EPO treated animals identified. RESULTS: The three libraries (control, hypoxia and rhEPO)contained 7.6, 8.1 and 7.1 million tags representing between 149,000 and 210,000 and 157,000 tag types (excluding singletons) respectively. Approximately 47,000 tag-types were unique to the EPO library. Most of these were at very low frequencies; however, 30 were present in at least 10 copies. DISCUSSION: We demonstrate that transcriptional signatures representing exposure to rhEPO can be characterized, and differentiated from the hypoxia response, in mouse blood, supporting the feasibility of using gene expression patterns in human blood as a basis for detection of EPO doping in athletes. Validation of these expression patterns in humans and analysis of inter-individual due to exercise, menstrual cycle, and time of blood draw is underway.

Sex, Aldosterone and Telomere Length in African American Adolescents. *H. Zhu, X. Wang, J. Thomas, K. Li, I. Stallmann-Jorgensen, G. A. Harshfield, Y. Dong* Dept Pediatrics, Med College Georgia, Augusta, GA.

Background: Telomeres are tandem repeats of TTAGGG at the ends of chromosomes that protect genomic integrity and progressively shorten with each cell division. Age, sex, body mass index (BMI), and renin-angiotensin-aldosterone (RAAS) system have been shown to affect the rate of telomere attrition in adults. However their effects on telomere length in youth, especially in African American youth remain unknown. Objectives: To evaluate the roles of age, sex, BMI and markers of RAAS system on telomere length in African American adolescents. Methods: One hundred and fifty nine African American adolescents (aged 17.0 1.3, 59% females) participated in the study. Relative telomere length (T/S ratio) was determined in buffy coat DNA samples using a rapid, real-time quantitative PCR method, which normalized telomere extension product (T) to the amount of extension product of a single copy gene (S), and standardized the results to a reference DNA sample. The relative T/S ratio was dichotomized at the median into two groups (long telomere group vs. short telomere group). In a subset of the sample including 61 individuals (61% females), plasma renin activity, angiotensin II and aldosterone were measured by competitive enzyme methods. Results: The relative T/S ratio was not associated with age or BMI in this age group. However, the percentage of female in the long telomere group is higher than in the short telomere group (70.0% vs. 48.1%, p=0.004). Moreover, plasma aldosterone levels were higher in the long telomere group as compared to the short telomere group (125.8 62.4 pg/ml vs. 90.4 29.2 pg/ml, p=0.036). Further adjustment of age and sex did not change the results (p=0.003 for the difference in sex and p=0.052 for the difference in aldosterone levels). No associations were found between renin, renin/aldosterone ratio and the relative T/S ratio. Conclusion: This is the first study to show that in African American adolescents, females tend to have longer telomere than males. Aldosterone appears to be positively correlated with telomere length in this group. The mechanism is not clear and warrants further investigation.

Proximal symphalangism in a neonate with a 13q12q14.3 deletion. *J. Kaplan, G. Enns, L. Hudgins* Stanford University, Stanford, CA.

Purpose: Proximal symphalangism is a rare autosomal dominant disorder characterized by ankylosis of the proximal interphalangeal joints, carpal and tarsal bone fusion, and conductive deafness. Mutations in both *NOG* (17q22) and *GDF5* (20q11.2) have been identified in this disorder as well as in other disorders involving abnormalities in joint morphogenesis. We describe a neonate with proximal symphalangism and radiohumeral synostosis who was found to have a deletion involving 13q12q14.3 by high-resolution chromosome analysis. The novel findings in this case prompted us to examine this region for proximal symphalangism candidate genes.

Methods: Genes in the deleted region were identified using OMIMs gene map. We then concentrated on genes with the potential to affect joint morphogenesis. We reviewed the literature for cases with similar deletions causing symphalangism and for studies involving candidate genes.

Results: To our knowledge, there are no reported cases of symphalangism associated with a deletion in 13q12q14.3. Candidate genes in this region include *FLT1*, a VEGF receptor that may play a role in osteoclastogenesis; *TNFSF11*, which is involved in the differentiation of osteoclasts; and *CHM1*, which encodes chondromodulin 1, a cartilage-specific glycoprotein that stimulates chondrocyte growth.

Conclusions: Based on this intriguing case, it is clear that there are genetic causes of proximal symphalangism that remain to be identified. Whereas *NOG* and *GDF5* mutations can result in increased osteoblast activity, it is certainly possible that defects in osteoclast activity may result in a similar synostosis phenotype. Haploinsufficiency of a gene in this region may be responsible for proximal symphalangism.

Polymorphisms of *HIF-1alpha* gene in Mexican patients with preeclampsia. *S. Nava-Salazar[1], E. N. Sánchez-Rodríguez[1], C. A. Mendoza-Rodríguez[1], P. Cruz-Cruz[2], M. A. Cerbón[1]* 1) Departamento de Biología, Universidad Nacional Autónoma de México, Ciudad Universitaria. Ciudad de México, Mexico; 2) Instituto Mexicano del Seguro Social, Hospital de Ginecología y Obstetricia No. 3 La Raza, Ciudad de México, México.

Preeclampsia is a pregnancy-specific syndrome characterized by new onset hypertension, proteinuria and edema. This disease is a considerable obstetric problem and significant source of maternal and neonatal morbidity and mortality. Although the pathophysiology of preeclampsia remains undefined, placental ischemia/ hypoxia are widely regarded as a key factor. The hypoxia inducible transcription factor, HIF-1alpha, is overexpressed in placentae from women with preeclampsia and contributes to the dysregulation of numerous genes related with trophoblast invasion. P582S and A588T are variants of HIF-1alpha that have been associated to high transcriptional activity in some types of cancer and are used as risk factor markers of the disease. In the present study, we explore the association of the allelic polymorphism P582S and A588T of HIF-1alpha gene and the association with prevalence of preeclampsia in Mexican population. The molecular analysis of P582S and A588T alleles was performed by PCR and direct sequencing. A case - control study of risk factors is currently undertaken. The preliminary results suggest that the allelic variants, P582S and A588T, present a similar frequency, in Mexican than other populations in the exploratory study. However, we are increasing the size of the sample to establish the prevalence of these polymorphisms and its possible association with preeclampsia in our population.

Multiple source genetic heterogeneity and population- and intra-familial tests of association for quantitative traits. *Y. Kim, A. J. M. Sorant, A. F. Wilson* Genometrics Section, IDRB, NHGRI, NIH, Baltimore, MD.

The presence of undetected genetic heterogeneity produces a loss of power in association studies. In this study, computer simulation was used to determine the effect of genetic heterogeneity due to multiple sources on tests of association between a quantitative trait and a causal SNP in both population and intra-familial study designs (unrelated individuals, and nuclear and extended families). G.A.S.P. [v3.3] was used to simulate a single quantitative trait in 3 sub-populations. In the association subpopulations (populations 1 and 2), the trait was based on a different SNP marker in each subpopulation; in the non-association subpopulation (population 3), the trait was due to a random effect. The heritability of the trait in the combined sample was fixed to be 0.05, and the subpopulation with the causal SNP (population 1) had a constant heritability of 0.025. In each simulation experiment, different proportions (100%, 50%, 30%, and 10%) from the association subpopulations (half from population 1 and half from population 2) were combined with the non-association subpopulation to produce samples with various degrees of genetic heterogeneity. ANOVA [SAS] was used to test for association in population-based tests of unrelated individuals. ASSOC [S.A.G.E.], FBAT and ROMP were used to perform intra-familial tests. The proportion of samples with a significant result was used to estimate the power at the causal SNP. Of the models considered, the power of the test of association, regardless of the method used, decreased dramatically as the proportion of the non-association subpopulation increased. The intra-familial ASSOC analysis had the greatest power in each scenario, followed by the population-based test, and then the intra-familial based ROMP and FBAT tests. In general, it appears that the presence of even a modest amount of genetic heterogeneity within a sample, regardless of the source, can cause a substantial loss of power to detect an association. Of the methods considered, the likelihood based intra-familial ASSOC test was the most robust to the presence of genetic heterogeneity.

Phenotypic spectrum of Microcephalic osteodysplastic primordial dwarfism type Majewski II caused by mutations in the Pericentrin (PCNT) Gene. *A. Rauch[1], C. T. Thiel[1], Y. J. Crow[2], A. J. van Essen[3], T. O. Goecke[4], L. Al-Gazali[5], K. H. Chrzanowska[6], H. G. Brunner[7], C. J. Curry[8], B. Dallapiccola[9], K. Devriendt[10], E. Kinning[11], A. Megarbane[12], P. Meinecke[13], R. K. Semple[14], A. Toutain[15], R. C. Trembath[16], R. Hennekam[16], A. Reis[1], H. G. Dörr[1]* 1) Inst Hum Genet, Univ Erlangen, Germany; 2) Leeds; 3) Groningen; 4) Düsseldorf; 5) Al-Ain; 6) Warsaw; 7) Nijmegen; 8) Fresno; 9) Rome; 10) Leuven; 11) Leicester; 12) Beirut; 13) Hamburg; 14) Cambridge; 15) Tours; 16) London.

In his book bird-headed dwarfs (1960) Helmut Seckel described a heterogenous group of individuals with variable degrees of primordial short stature and microcephaly. In 1982 Majewski and colleagues reviewed cases reported as Seckel syndrome and distinguished three types of osteodysplastic primordial dwarfism from bona fide Seckel syndrome. Using positional cloning, we recently identified biallelic loss-of-function mutations in the pericentrin (PCNT) gene resulting in disorganized mitotic spindles, premature sister chromatid separation and missegregation of chromosomes to cause microcephalic osteodysplastic primordial dwarfism type Majewski II (MOPD II) (Rauch et al. 2008, Science 319:816-9). Since this distinct entity is commonly not well known we report here on the clinical spectrum and natural history of 26 MOPD II patients with biallelic pericentrin mutations in more detail. Adults with this condition belong to the shortest of the short having a height of about 100 centimeters. We observed that deviation from the mean increased with age, with average birth weight of -3.5 SDS, average height at last investigation of -9.6 SDS and average head circumference of -8.9 SDS. Although most of our patients had an IQ within the low-normal range, 6 of 26 were moderately to severely retarded, due to cerebral complications such as multiple infarctions or cerebral bleedings commonly associated with Moyamoya disease (10/26 patients). Despite the fact that only 3 of our patients were older than 12 years, 4 had already type 2 diabetes and dyslipidemia. Three patients died at age 9, 11 and 14 years from subarachnoid or aneurysm bleeding, and one died at age 3 years from cardiomyopathy.

Identification of six candidate long-range cis-regulatory elements upstream to *NR0B1* (*DAX1*) using 3C technique. *M. Nowak[1,2], P. Stankiewicz[1,2]* 1) Dept of Medical Genetics, Institute of Mother and Child, Warsaw, Poland; 2) Dept. of Molecular & Human Genetics, Baylor College of Medicine, Houston TX.

Defects in long-range regulatory elements have been shown as a mechanism underlying genetic diseases, with most of them involving dosage-sensitive genes. NROB1, the nuclear receptor 0B1 (DAX1) is essential for the development and functioning of hypothalamus-pituitary-gonadal axis. Deletion of NR0B1 in Xp21.2 results in congenital adrenal hypoplasia (AHC), whereas NR0B1 duplication in 46,XY individuals leads to gonadal dysgenesis and a female phenotype (dosage sensitive sex reversal, DSS). We have reported recently a 257,782 bp deletion, 11 kb upstream to *NR0B1* in a 21-year-old 46,XY female manifesting primary amenorrhea, a small immature uterus, gonadal dysgenesis, and apparent normal adrenal function and suggested a loss of regulatory sequences resulting in position effect up-regulation of DAX1 expression. To verify this hypothesis, we have analyzed 300 kb region upstream to *NR0B1* using chromosome conformation capture (3C) technique in a lymphoblastoid and HeLa cell lines. In lymphoblasts, we have identified four sequences located ~81 kb, ~111 kb, ~121,5 kb, and ~176 kb 5 to *NR0B1*, showing high intensity physical interaction with *NR0B1*, and two sequences ~95.7 kb and ~187.4 kb 5 to *NR0B1* with weaker intensity interactions with the gene. Conversely, in HeLa cells, the ~95.7 kb and ~187.4 kb sequences showed the highest intensity, the ~111 kb, ~121,5 kb, and ~176 kb intermediate intensity, whereas the ~81 kb did not show physical proximity with NR0B1, indicating cell line dependent variety of interactions. All the identified elements correspond to, or are in close proximity to evolutionarily conserved nongenic sequences described previously. Our results confirm the hypothesis that these long-range upstream elements have regulatory impact on *NR0B1* expression.

Genetic association study of height growth and timing of puberty in the Northern Finland Birth Cohort 1966. *U. Sovio[1], A. J. Bennett[2], N. J. Timpson[2,3], J. Haukka[4], I. Millwood[1], J. Molitor[1], E. Widen[5], L. Peltonen[5,6], M. I. McCarthy[2], M.-R. Jarvelin[1,7]* 1) Imperial College London, UK; 2) University of Oxford, UK; 3) Bristol University, UK; 4) National Public Health Institute, Helsinki, Finland; 5) University of Helsinki, Finland; 6) Broad Institute of Harvard and MIT, Cambridge, USA; 7) University of Oulu, Finland.

Adult height is a complex, yet highly heritable trait. Recent findings from GWA studies suggest that many common variants are associated with this trait. However, it is unclear how these variants are involved in height growth throughout childhood. We derived peak height velocity (PHV) and pubertal timing parameters from longitudinal height growth data to test their association with known height variants. The study sample consisted of N=3,538 singletons from the Northern Finland Birth Cohort 1966 with genotype data and on average 20 height measurements/person at 0-20 years. PHV in infancy was derived from sex-specific 4-parameter Reed1 models and regressed on each SNP, weighted by the number of measurements, adjusted for sex and population structure. PHV in puberty, age at growth spurt take-off and age at PHV in puberty were derived from sex-specific 8-parameter JPA2 models and regressed on each SNP. We replicated 23 of the 43 signals found in GWA scans with adult height in this sample (p0.05). Seven SNPs in HHIP, DLEU7, GDF5, SF3B4, LCORL and HIST1H1D showed association with PHV in infancy. Five SNPs in SOCS2, SF3B4, C17orf67, CABLES1 and DOT1L were associated with PHV in puberty and two SNPs in SOCS2 and C6orf106 with timing of puberty. In this sample, few genetic variants associated with adult height had a measurable effect on PHV in infancy or puberty. However, statistical power was limited to 80% to detect a per allele effect size of 7% SD in infancy and 8% SD in puberty, whereas for adult height we had similar power to detect an effect size of 6% SD (0.6cm) at level p0.05. GWA studies on height growth parameters will help identify genomic regions affecting height growth velocities at different stages of growth and timing of puberty.

Follow-up of 22 Prader-Willi syndrome patients. *L. M. J. Albano[1], C. R. S. Silva[1], M. C. Varela[2], C. P. Koiffmann[2], D. Bertola[1], R. S. Honjo[1], I. M. Furquim[1], C. A. Kim[1]* 1) Pediatrics, Instituto da Criança, São Paulo, São Paulo, Brazil; 2) Insituto de Biociências, University of São Paulo, São Paulo, Brazil.

Prader-Willi syndrome (PWS) is a genetic disorder characterized by neonatal hypotonia, mental retardation, behavioral abnormalities, dysmorphic features, hyperphagia and progressive obesity. The expression of imprinted paternal genes is abolished, mainly due to a paternal deletion within 15q11-q13 (70-75%). Maternal uniparental disomy of this same region, a defect in the imprinting center, and translocations occur in 20-25%, 2-5%, and 1%, respectively. We studied 22 PWS cases (10F:12M) evaluated at our Unit since 1996 (12 years) that fulfilled the clinical criteria consensus, confirmed by methylation analysis. All of them were sporadic cases and the age at the diagnosis ranged from 7 days to 16 years. The molecular PWS diagnosis was established before 1 year of age in seven patients (32%). Low birth weight (<2,500g) was noticed in 7/21 (32%); hypotonia improved over time and was present at birth in all cases; reduced fetal movements were referred in 10/14 cases (71%); assisted delivery (cesarean section) was required in all of them. Hypogonadism and poor or absent sucking was observed in all patients and 7/16 (44%) required gavage feeding. In 4/11 (36%) temperature instability was referred and 2 presented hip dislocation. All patients referred some infectious disease, mainly respiratory. Obesity was noted in all patients older than 3 years of age and in two of them it was present in a more precocious age. Growth hormone therapy was performed in 7/13 patients (54%), all after 6 years of age but one, who started at 1 year of age. Mental retardation was referred in all patients. Six patients died at an age that ranged from 9 to 19y, due to cardio-respiratory problems. One patient was submitted to a bariatric surgery at 10 years of age. Dysmorphic features, as well as behavioral problems, were noticed in more than 75% of the patients. All G-banded karyotypes were normal. All cases presented a positive methylation test for PWS syndrome: 14 (64%) showed a microdeletion, 5 (23%) a maternal uniparental disomy and in 3 (13%) the genetic mechanism could not be established.

-Nicotinic Acetylcholine Receptor (CHRNA3/5) Polymorphisms Influence Risk of Chronic Obstructive Pulmonary Disease (COPD). *SG. Pillai[1], D. Ge[2], G. Zhu[1], X. Kong[1], KV. Shianna[2], A. Need[2], C. Hersh[3], P. Bakke[4], A. Gulsvik[4], A. Ruppert[5], K. Carlson[6], A. Roses[2], W. Anderson[1], S. Rennard[7], D. Lomas[8], EK. Silverman[3], DB. Goldstein[2], ICGN & ECLIPSE Investigators* 1) Genetics, GlaxoSmithKline, RTP, NC; 2) Duke University, Durham, NC; 3) Brigham and Women's Hospital, Boston, MA; 4) University of Bergen, Norway; 5) GRC, Munich, Germany; 6) University of Oslo, Norway; 7) University of Nebraska, Omaha, NE; 8) CIMR, Cambridge, UK.

Recent genome-wide association studies (GWAS) have identified the CHRNA3/5 locus on chromosome 15 as a major genetic risk factor for lung cancer. This GWAS and subsequent replication studies in four COPD populations found that the same locus associates with COPD, highlighting the public health importance of this locus in smoking related diseases. The GWAS was conducted in a case-control cohort from Bergen, Norway (823 cases and 810 controls) and evaluated the top 100 SNPs in a family-based study, the International COPD Genetics Network (ICGN, 1891 subjects from 606 pedigrees). The SNPs that showed replicated associations were further evaluated in 389 subjects from the National Emphysema Treatment Trial (NETT) and 472 controls from the Normative Aging Study (NAS). The SNPs with genome-wide significance were then evaluated in the ECLIPSE (Evaluation of COPD Longitudinally to Identify Predictive Surrogate End points) cohort to identify association with COPD-related phenotypes. In the Bergen cohort, 3 SNPs on chromosome 5 reached genome-wide significance, but showed only nominal association in the replication studies. Two SNPs in the CHRNA3/5 locus, rs8034191 and rs1051730, showed unambiguous replication in the ICGN and in the NETT/NAS studies with a combined p value of 8.31×10^{-13} and 1.93×10^{-11}. Analysis in the ECLIPSE COPD subjects showed association of the CHRNA3/5 SNP (rs1051730) with airflow obstruction (FEV1/FVC; $p=7 \times 10^{-4}$) and high resolution CT-defined emphysema ($p=1.3 \times 10^{-5}$). These results suggest the possibility of multiple functional polymorphisms in the region or a single polymorphism with wide phenotypic consequences, potentially mediated through an effect on smoking behavior.

Coronary Artery Disease Risk Locus at 9p21.3 Alters *ANRIL* Expression and Modulates Cell Proliferation Pathways. *O. Jarinova[1], A. Stewart[1], P. Lau[1], T. Naing[1], R. Roberts[1], G. Wells[1], C. Buerki[2], B. McLean[2], R. Cook[2,3], J. Parker[4], R. McPherson[1]* 1) University of Ottawa Heart Institute, Ottawa; 2) Med BioGene Inc., Vancouver; 3) University of British Columbia, Vancouver; 4) Expression Analysis, Durham, NC.

We and others recently identified a novel intergenic locus on chromosome 9p21, which remained robustly associated with coronary artery disease (CAD) risk upon replication in numerous independent samples. The risk locus spans 58 kb and encompasses multiple single nucleotide polymorphisms in tight linkage disequilibrium. Approximately 25% of Caucasians carry 2 copies of the risk allele and have a 1.5 fold increased risk for CAD, independently of all known risk factors, implying a novel biological pathway relevant to atherosclerosis. The risk locus lies centromeric to the alternatively spliced cyclin dependent kinase inhibitors, *CDKN2A* ($p14^{ARF}$, $p16^{INK4A}$) and *CDKN2B* ($p15^{INK4b}$) which function in cell proliferation, aging and apoptosis. We have determined the effects of genetic variation at the 9p21.3 locus on gene expression profiles in whole blood RNA. Since lymphocyte gene expression is altered in a number of disease states and in response to various medications, only healthy control subjects homozygous for the 9p21.3 reference or alternative alleles were studied. Pathway analysis revealed upregulation of gene sets regulating cell proliferation in carriers of the risk allele. It is notable that the 9p21 risk locus overlaps a newly annotated antisense non-coding RNA (*ANRIL*) which spans 126.3 kb and overlaps at its 5 end with *CDKN2B* ($p15^{INK4b}$). Quantitative real time PCR revealed that expression of the long variant of *ANRIL* was decreased by 1.3 fold whereas expression of the short *ANRIL* variants was increased by 2.2 fold in subjects homozygous for the risk allele and this difference was associated with a decrease in relative copy numbers of transcripts involved in cell proliferation, namely *CDKN2A*, *CDKN2B* and *GAPDH*. We hypothesize that the 9p21.3 risk allele promotes atherosclerosis by regulating the alternative splicing of *ANRIL*, which in turn alters the expression of genes controlling cell proliferation pathways.

Genome-Wide Association Study in Parkinsons Disease: Identification of Strong Associations with SNCA and MAPT. *S. Scholz[1,2], C. Schulte[3], P. D. Genetics Consortium[1,2,3,4,5,6,7]* 1) Laboratory of Neurogenetics, National Institute on Aging, Bethesda, MD, USA; 2) Department of Molecular Neuroscience, Institute of Neurology, Queen Square, London, UK; 3) Hertie Institute of Clinical Brain Research, Tuebingen, Germany; 4) Department of Medical Genetics, University of Tübingen, Tübingen, Germany; 5) Institute of Epidemiology, Neuherberg, Germany; 6) Institute of Medical Biometrics, Informatics and Genetics, University of Bonn, Bonn, Germany; 7) Department of Neurology, University of Lübeck, Lübeck, Germany.

Background: Parkinsons disease (PD) is the second most common neurodegenerative disorder. The etiology of PD is largely unknown. Methods: We performed a genome-wide association study in PD in 1713 cases and 3978 neurologically normal controls. We genotyped each individual on Illumina genotyping chips (Illumina humanhap550 or Illumina humanhap240S + Illumina humanhap317K) yielding over 550,000 genotypes/individual. SNPs with minor allele frequencies (MAF) 1%, Hardy-Weinberg Equilibrium $p > 0.001$ and genotype call rates 98% were included in the analysis (463,187 SNPs). We performed association tests using the additive model in PLINK software. Results: We identified clear association exceeding Bonferroni correction level for the following loci: SNCA (p=5.69E-9, OR=1.27) and MAPT (p=4.50E-8, OR=0.74). Conclusions: SNCA has previously been implicated with PD in familial forms of disease, whereas MAPT mutations are known to be involved in a number of neurodegenerative disorders. Our data suggest that genetic variants at the SNCA and MAPT locus play a critical role in the pathogenesis of PD. The PD Consortium consists of: D. Berg, M. Bonin, E. Caliskan-Erle, G. Deuschl, J.R. Gibbs, C. Gieger, J. Hardy, D. Hernandez, T. Illig, C. Klein, R. Krüger, P. Lichtner, C. Paisan-Ruiz, S. Poths, O. Riess, S. Scholz, C. Schulte, M. Sharma, J. Simon-Sanchez, H.E. Wiechmann, T. Wienker, T. Gasser, A.B. Singleton.

Allelic variations upstream of the T1R3 gene correlate with sucrose sensitivities in humans. *A. Fushan[1], J. Slack[2], C. Simons[2], A. Manichaikul[1], D. Drayna[1]* 1) NIDCD, National Institutes of Health, Rockville, MD; 2) Givaudan Flavors Corp., Cincinnati, OH.

Members of the human TAS1R class of taste-specific G protein-coupled receptors have been proposed to function in combination as heterodimeric sweet taste receptors. TAS1R2/TAS1R3 heterodimers recognize sweet taste stimuli. We hypothesized that allelic variation of the TAS1R3 receptor gene can be one of the mechanisms determining differences in individual sensitivities to sweet compounds. To test this hypothesis, we analyzed sucrose and sucralose sensitivities of 130 unrelated human volunteers aged 20 - 55 years, using a sorting test and subsequent cumulative R-index score. Then, we examined the genomic sequence of the T1R3 gene in these individuals including exons, introns, and upstream and downstream regions, to survey all polymorphisms that could affect amino acid composition, RNA splicing, or potential regulatory regions. Quantitative trait analysis revealed significant association (adjusted $p<0.01$) between phenotypes and SNPs located in the genomic region 2000 bp upstream of T1R3 coding sequence. Together the associated alleles account for approximately 8% of the total variation in sweet sensitivity in our subject population. Bioinformatic analysis predicts these polymorphism are located in the regions of transcription factor binding sites, suggesting that differences in expression levels of T1R3 are a significant factor determining human sweet sensitivity in vivo.

Candidate gene analysis reveals genetic pathways associated with a persistent pain disorder. *S. Smith[1], M. Siddiqi[1], V. Miller[1], D. Gibson[1], R. Arunasalam[1], P. Kasravi[1], G. Slade[2], A. Neely[1], E. Bair[1], W. Maixner[1], L. Diatchenko[1]* 1) Sch Dentistry, Univ North Carolina, Chapel Hill, NC; 2) Sch Dentistry, Univ Adelaide, Adelaide, Australia.

Although evidence supports a heritable basis for pain conditions such as temperomandibular joint disorders (TMJD) and fibromyalgia, few genetic determinants for persistent pain have been identified. To explore the genetic basis of chronic pain conditions, we have created a genotyping chip comprised of 3295 single nucleotide polymorphisms (SNPs), representing over 300 genes known to influence pain pathways. This panel was designed to assay SNPs most likely to regulate gene expression and function, employing Paralleles MIP technology. In the first use of this platform, 200 Caucasian females with a history of chronic TMJD were recruited as cases at a multidisciplinary pain clinic, with corresponding controls. Subjects underwent a comprehensive battery of nociceptive and psychosocial assessments. DNA from donated blood was genotyped using our pain candidate gene chip. Statistical associations between genetic markers and disease phenotypes were examined using PLINK software. Genotypic trend tests were employed to determine associations between candidate genes and disease status, and linear regression was used for quantitative sensory and affective traits. Numerous polymorphisms were significantly implicated in persistent TMJD or its putative mechanisms, such as stress modulation and nociceptive neurotransmission. Associated odds ratios for common (MAF>0.05) risk alleles for TMJD ranged up to 2.2. Haplotype analysis further revealed multilocus effects contributing to genetic variability in these traits. The results of this study were compared with associations previously revealed in a separate incidence sample of 186 initially disease-free subjects; Several risk genes, including genes for adrenergic and opioid receptors, were replicated in this study, supporting the likelihood that they are true associations. Using a candidate gene approach, we successfully implicated a number of pain-relevant genes and pathways in susceptibility to a persistent pain disease.

Mapping successful aging phenotypes to chromosome 2q14 in the Amish. *W. K. Scott[1], P. J. Gallins[1], J. L. McCauley[1], L. Jiang[2], M. Creason[1], L. Caywood[1], D. Fuzzell[2], C. Knebusch[2], C. E. Jackson[3], J. R. Gilbert[1], M. A. Pericak-Vance[1], J. L. Haines[2]* 1) University of Miami, Miami, FL; 2) Vanderbilt University, Nashville, TN; 3) Scott & White, Temple, TX.

Successful aging (SA) is a multi-dimensional phenotype involving preservation of cognitive ability, physical function, and social engagement throughout the lifespan. Several quantitative components of the SA phenotype (longevity, upper and lower extremity function, and cognitive ability) are heritable. The Indiana and Ohio Amish are genetically and socially isolated from the surrounding communities and maintain homogeneous lifestyles. These conditions make the Amish an optimal population for identifying loci associated with successful aging. We collected DNA along with subjective and objective measures of function, cognition, life satisfaction, and social support on more than 672 Amish individuals over age 65. Using the Anabaptist Genealogy Database (AGDB), we grouped all individuals into a single 11-generation pedigree. Subsequently, we performed a genome-wide linkage screen of 5,944 single nucleotide polymorphisms (SNPs). We utilized the GREFFA program to create 7 sub-pedigrees for linkage and association analysis. Analysis of the SA phenotype (48 individuals over age 80 who were cognitively intact, not depressed, satisfied with life, little self-reported limitation in activities of daily living or musculoskeletal function, in the top 1/3 of the sample in lower-extremity physical function, and having adequate social support) identified 11 SNPs with 2-point lod scores greater than 1.0 and association p-values less than 0.05. Analyses of quantitative measures of physical function (grip strength and lower extremity function) were also performed. Notably, the SNPs with the strongest evidence for linkage and association with both the overall SA phenotype (rs2056282: lod=2.6, p=0.026) and quantitative measure of lower extremity function (rs1712865: lod=2.4, p=0.05) co-localized to a 7 Mb region of chromosome 2q14. These results suggest that 2q14 harbors a locus promoting successful aging, whose effect may influence lower extremity function (balance, gait, and mobility) in older adults.

Elucidating a novel gene associated with Inherited Myoclonus Dystonia. *T. J. Read[1,2], D. A. Grimes[2,3], D. E. Bulman[1,2,3]* 1) Dept. of Biochemistry, Genetics, University of Ottawa, Ottawa, Ontario, Canada; 2) Ottawa Health Research Institute, Ottawa, Ontario, Canada; 3) Division of Neurology, Dept. of Medicine, University of Ottawa, Ottawa, Canada.

Inherited Myoclonus Dystonia (IMD) is an autosomal dominant disease with high but incomplete penetrance and is characterized by both involuntary myoclonic jerks and dystonic posturing. We have found that mutations within the epsilon sarcoglycan (SGCE) gene on chromosome 7q21 are associated with IMD in 30-40% of affected individuals in 31 families studied, supporting the basis for genetic heterogeneity. Novel mutations have been found in the SGCE gene by screening these families for point mutations and large deletions/duplications through the use of sequencing and MLPA exon dosage analysis, respectively. A 10cM genome wide linkage analysis of a large Canadian family provided significant LOD scores for microsatellite markers within the 18p11 region, now designated as the DYT 15 locus. Further haplotype analysis has narrowed a non-recombinant region associated with the disease phenotype to a 3.18 Mb region in this locus. Since the current understanding of Myoclonus Dystonia is poor, it is difficult to predict genes that could be responsible for IMD. Sarcoglycans are essential constituents of the dystrophin-glycoprotein complex and are involved in linking the extracellular lamanin matrix to the actin filaments within the cytoplasm, therefore focus is given to the examination of potentially related structural genes that are expressed in the brain. By analyzing such candidate genes in a panel of affected individuals, we believe that a novel gene will be elucidated and provide insight into the mechanism of Inherited Myoclonus Dystonia.

The evolutionary history of lactase persistence in Africa. *A. Ranciaro[1,2], F. Reed[3], J. Hirbo[1], K. Powell[4], M. Osman[5], H. Muntaser[5], O. Sabah[6], A. Fremont[7], S. A. Tishkoff[1,8]* 1) Dept Genetics, Univ Pennsylvania, Philadelphia, PA; 2) University of Ferrara, Italy; 3) Department of Evolutionary Ecology, Max Planck Institute for Evolutionary Biology, Plön, Germany; 4) University of Maryland, College Park, MD; 5) Institute of Endemic Diseases, University of Khartoum, Sudan; 6) Kenya Institute of Medical Research, Nairobi, Kenya; 7) Museum National d'Histoire Naturelle, Centre National de la Recherche Scientifique, Paris, France; 8) Department of Biology, University of Pennsylvania, Philadelphia, PA.

In most individuals, the ability to digest lactose, the sugar present in milk, declines rapidly after weaning because of decreasing levels of the enzyme lactase (lactase-phlorizine hydrolase, LPH) in the small intestine. However, there are individuals who maintain the ability to digest milk into adulthood due to a genetic adaptation in populations that have a history of pastoralism. In order to identify variants associated with the lactase persistence (LP) trait and to study the evolutionary history of LP in Africa, we resequenced 1.7 kb of intron 9 and 3.3 kb of intron 13 of the *MCM6* gene (associated with LP in Europeans), and 2.2 kb of the promoter region of the *LCT* gene in 821 Africans and a comparative sample of 138 Middle Easterners, 34 Europeans, and 23 Chinese. We analyzed genotype/phenotype associations in >500 individuals for which we measured lactase activity using a lactose tolerance test and identified three variants associated with the LP trait in Africans (G/C-14010, T/G-13915, and C/G-13907). We identify a strong signature of recent positive selection in several East African pastoralist groups. Microsatellite haplotype analysis was used to reconstruct the origin and spread of the LP associated variants in Africa. Our results indicate that mutations associated with LP arose independently in ethnically and geographically African populations. Additionally, we find evidence for an East African origin for the spread of pastoralism into South Africa.

Genome wide linkage analysis using SNPs in high performance distributed environments. *A. Calabria[1], D. Di Pasquale[1], A. Orro[1], G. Trombetti[1], E. Salvi[1,2], L. Milanesi[1]* 1) Institute for Biomedical Technologies, National Research Council, Segrate, Milano, Italy; 2) Dept of Science and Biomedical Technologies, Università degli Studi di Milano, Italy.

The problem of Linkage Analysis is NP-hard, and the computational cost and memory requirements of the major algorithms proposed in literature grows exponentially with pedigree size and markers' number. Implementations of these algorithms reflect such limitations, making analyses of medium/large data sets very hard on a single CPU. The aim of the present work is to enable the use of high performance computing infrastructures, such as clusters and grid infrastructures, for the execution of linkage analysis on large data sets, especially for the case of SNPs-based analysis, without the need to own dedicated HPC resources. The approach used relies on the EGEE grid framework and grants an easy web access to the GRID platform, hence requiring only very basic informatics knowledge. Some of the most used linkage analysis software have been ported to clusters and the gLite EGEE Grid environments, and a system has been designed and implemented in order to realize a parallel approach for the linkage analysis. The method adopts inputs' splitting to achieve the best balance for computational time/memory cost on the basis of the specific algorithm adopted by the linkage program, and subsequently runs the processes in parallel. The system provides a web user interface which allows an easier approach to linkage analysis softwares on grid and cluster environments. Below the interface, the workflow progress is supported by a reliable and scalable grid system facility called VNAS, which monitors and manages grid interactions (submission and job retrieving). Tests results show that when reaching the computational limits in data set size there is a real benefit in the use of our implementation: mean improvements of about 65% in computational time compared to a single computer execution was experienced adopting Genehunter with 26 individuals and 80 markers. Increasing the number of individuals some computations, still completed successfully on the Grid, but resulted infeasible on a desktop PC due to memory overflow.

Bone Density, Sex Hormone Levels and Spinal Abnormalities in Vascular Ehlers-Danlos Syndrome Patients. *N. L. Commins, N. Obeng-Adjei, B. F. Griswold, L. J. Sloper, N. B. McDonnell* National Institute on Aging, Baltimore, MD.

Vascular Ehlers-Danlos Syndrome (VEDS) is a hereditary disorder of connective tissue that arises from mutations in COL3A1 . Patients with VEDS are susceptible to bowel and arterial rupture, and joint laxity is seen. Bone density, sex hormone levels and spine abnormalities have not been studied systematically. We evaluated these features in twenty-four patients with genetically confirmed VEDS using dual energy x-ray scans and magnetic resonance imaging, and laboratory measurements of sex hormones. Osteopenia (17/24)and osteoporosis (6/24) were commonly seen in vascular EDS subjects, all affected were over 18 years of age. Spinal abnormalities including dural ectasia (18/24), disc desiccation (14/24), disc disease (12/24), disc protrusions or bulges (12/24) and facet arthrosis (4/24) were also common. Abnormal levels of testosterone (5/24) and sex hormone binding globulin (13/24) occurred frequently among the subjects. Patients with low testosterone levels showed a high occurrence rate of osteopenia and osteoporosis. It was noted that there were particular hot spots of spinal abnormalities including disc desiccation, disc protrusions and bulges at L1-L2 and L5-S1, and C3-C4 and C6-C7. It was also noted that dural ectasia and disc disease occurred with higher frequency among women and disc desiccation and disc protrusions/bulges occurred with higher frequency among men. Although patients with low testosterone levels had low bone densities as expected, many patients with normal testosterone or estrogen levels also showed low bone densities (11/24). Abnormalities of sex hormones, bone density, and frequent incidence of spine pathology are previously unrecognized complications of VEDS.

Convergent patterns of association between phenylalanine hydroxylase variants and schizophrenia in four independent samples. *L. McClain[1], M. Talkowski[1], T. Allen[3], D. Bradford[4], M. Calkins[5], N. Edwards[6], R. Go[8], R. Gur[5], R. Gur[5], G. Kirov[7], D. Toncheva[11], J. Kwentus[9], P. Lyons[2], H. Mansour[1], J. McEvoy[3], M. O'Donovan[7], J. O'Jile[9], M. Owen[7], A. Santos[10], V. L. Nimgaonkar[1]* 1) University of Pittsburgh, Psychiatry, Human Genetics, Pediatrics; 2) University of VA, Neurology; 3) Duke University Medical Center-John Umstead Hospital; 4) Morehouse School of Medicine, Psychiatry; 5) University of Pennsylvania, Psychiatry; 6) University of Tennessee, Psychiatry; 7) Cardiff University School of Medicine, Psychological Medicine; 8) University of Alabama Birmingham, Psychiatry, Behavorial Neurobiology, Epidemiology; 9) University of Mississippi, Psychiatry and Human Behavior; 10) Medical University of South Carolina, Psychiatry and Behavioral Sciences; 11) University Hospital, Maichin Dom, Medical Genetics.

Recessive mutations in phenylalanine hydroxylase (PAH) predispose to phenylketonuria (PKU) in conjunction with dietary exposure to phenylalanine. Previous linkage and association studies suggested PAH variations could confer risk for schizophrenia. We analyzed 15 PAH tag SNPs and 3 rare exonic variations among four independent samples from the US and Bulgaria (n=5,414), including three Caucasian cohorts (US: 1. 260 trios, 2. 230 cases/474 controls, Bulgaria: 3. 659 trios) and an African-American sample (464 families, 401 controls). Initial analyses of the US Caucasian samples revealed significant associations with 5 SNPs (uncorrected $p<0.05$); most notably allele (G) of rs1522305 ($p=0.006$). This SNP was independently replicated in 659 Bulgarian trios ($p=0.015$). A non-significant trend was also observed among the African American families. Combined analyses across samples were significant for this allele (2=23.28, 8 df, $p=0.003$). Case-control differences were also noted among African Americans with the common allele of L321L ($p=0.047$, OR=1.46). Our analyses suggest several PAH variations could be risk factors for SZ. R.Savage8; L. Georgieva7; K.V. Chowdari1; G. Vockley1; J. Wood1; B. Devlin1;.

Lineage-specific and acquired epigenetic changes in diffuse large B-cell lymphoma. *X. Wang[1], T. C. Greiner[2], B. L. Pike[1], D. J. Weisenberger[3], K. D. Siegmund[4], P. W. Laird[3], J.-B. Fan[5], J. G. Hacia[1]* 1) Department of Biochemistry and Molecular Biology, University of Southern California Los Angeles, CA, USA; 2) Departments of Pathology and Microbiology University of Nebraska Medical Center Omaha, NE, USA; 3) Department of Surgery University of Southern California Los Angeles, CA, USA; 4) Department of Preventive Medicine University of Southern California Los Angeles, CA, USA; 5) Illumina, Inc. San Diego, CA, USA.

In an initial epigenetic characterization of diffuse large B-cell lymphoma (DLBCL), we evaluated the DNA methylation levels of over 1,500 CpG islands using the Illumina GoldGate BeadArray methylation platform. Sixty-seven CpG islands showed significant methylation in over 85% of tumors. Interestingly, the methylation levels of CpG islands proximal to 31 genes differed between the activated B-cell-like (ABC-DLBCL) (10 cases) and germinal center B-cell-like (GCB-DLBCL) (14 cases) subtypes. In parallel, we conducted DNA methylation analyses on normal B-cell precursors of ABC-like and GCB-like tumors isolated from normal human plasma and lymph nodes by fluorescence-activated cell sorting (FACS). By comparing these methylation profiles to that of the corresponding tumors, we identified tumor-specific DNA methylation that was acquired during carcinogenesis and lineage-specific DNA methylation that is present in normal B-cell precursors. In addition, we compared the methylation and expression status of 161 genes proximal (within 500-bp) to the methylation assays. We frequently observed that hypermethylated CpG islands are proximal to genes that are expressed at low or undetectable levels in tumors. However, many of these same genes were also poorly expressed in DLBCL tumors where their cognate CpG islands were hypomethylated. Lastly, the moderate expression of several genes proximal to hypermethylated CpG tracts suggests that DNA methylation assays are not always accurate predictors of gene silencing. Overall, further investigation of the highlighted CpG islands as potential clinical biomarkers is warranted.

Cognitive Function in Autosomal Recessive Polycystic Kidney Disease. *E. J. Johnson[1], E. Wiggs[2], J. Bryant[1], A. Garcia[1], D. Adams[1], M. Tuchman[1], L. Guay-Woodford[3], W. A. Gahl[1,4], M. Gunay-Aygun[1,4]* 1) Medical Genetics Branch, National Human Genome Research Institute , Bethesda, MD; 2) National Institutes of Health, Clinical Center, Bethesda, MD; 3) University of Alabama, Birmingham, Al; 4) Office of Rare Disorders, DHHS, Washington, DC.

Autosomal Recessive Polycystic Kidney Disease/Congenital Hepatic Fibrosis (ARPKD/ CHF), the most common form of PKD in children, is characterized by progressive cystic degeneration of the kidneys resulting in chronic renal insufficiency and congenital hepatic fibrosis complicated by portal hypertension (PH). Although the central nervous system is not primarily involved, certain characteristics of ARPKD/CHF, might predispose affected children to developmental delay in early childhood and/or affect their cognitive function later in life. These complications include oligohydramnios, hypoplastic lungs requiring mechanical ventilation, severe systemic hypertension from birth, chronic renal insufficiency and portosystemic shunting. In our ongoing NIH study on ciliopathies (ClinicalTrials.gov, number, NCT00068224), we evaluated 60 ARPKD/CHF patients (age 12.3 + 11.9 years) with at least 1 pathogenic PKHD1 mutation. Developmental history was normal in 44 of 60 patients while 15 had mild to moderate delays in the areas of gross and/or fine motor and/or expressive speech. One patient, who required 17 amniotic fluid infusions, had severe global delays. Twenty-one of the 60 ARPKD/CHF patients (11 females, 10 males, mean age 10.1 + 5.7 years) underwent either the Wechsler Intelligence Scale for Children- Fourth Edition (WISC-IV) or the Wechsler Adult Intelligence Scale- Third Edition (WAIS-III) administered by a psychologist with extensive experience. Scores in the areas of verbal comprehension, perceptual reasoning/organization, working memory, and processing speed, were at the 65th + 15, 55th + 30, 51st + 30, and 43rd + 17 centiles, respectively. Mean full scale intelligence quotient (FSIQ) was 102 + 15 (at 58th + 28 centile). There was no correlation between FSIQ and creatinine clearance or platelet count as a measure of PH. Our results suggest that most ARPKD/CHF patients have normal cognitive development.

The NCBI dbGaP database of genotypes and phenotypes provides resources for genome-wide association studies.
S. Sherry, M. Feolo, Y. Jin, M. Kimura, K. Tryka, R. Bagoutdinov, J. Paschall, L. Hao, A. Kiang, L. Phan, N. Popova, S. Pretel, L. Ziyabari, M. Lee, Y. Shao, Z. Wang, M. Xu, M. Kholodov, S. Shevelev, J. Ostell NCBI, NIH, Bethesda, MD.

The National Center for Biotechnology Information (NCBI) offers investigators an unprecedented wealth of data for research through the dbGaP database of genotypes and phenotypes. Launched in December 2006, dbGaP (http://view.ncbi.nlm.nih.gov/dbGaP) assembles and redistributes comprehensive data sets from large scale association studies to support research problems in genome-wide disease association, population structure, haplotype analysis, and genetic epidemiology. dbGaP serves the research community in the dual roles of repository-of-record for published research, and as an information discovery space that provides summary reports, on-line documentation, browsers to explore association results on the genome, and connections to other information resources. For authorized investigators, dbGaP distributes de-identified, individual-level phenotype and genotype data for use in investigator-specified research projects in accordance with the NIH GWAS policy (http://grants.nih.gov/grants/gwas). dbGaP provides summary-level and individual-level data for 29 studies: longitudinal (including NHLBI Framingham SHARe, n=4), family-based not longitudinal (n=1), case-control (including Macular Degeneration and GAIN studies, n=16, association-results with no individual level data n=3), case-only (n=2), control-only (phenotyped normal controls n=1, genotyped HapMap samples n=2). In total, these studies provide individual-level data for over 30,000 phenotype measures and 22.4 billion genotypes across 55,109 study participants (mean participants per study = 2705). 188 investigators have been approved for access to dbGaP, and 146 of them have downloaded at least one study component with de-identified, individual-level data. The presentation will cover content submission, curation, accessioning, public summary-level measures, organization and request process for obtaining individual-level data, and a demonstration of the supporting tools and browsers that support exploration of submitted association results.

First Report of a Partial Duplication of Xp at prenatal Diagnosis. *R. Habibian[1], J. C. Wang[1], D. Liang[1], H. Makary[1], K. Troxell[2], A. Hajianpour[1]* 1) Cytogenetics Laboratory, Genzyme, Monrovia, CA; 2) University of Missouri Department of Child Health Mercy St. John's Hospital, Springfield, MO.

Duplication of Xp in conjunction with a normal Y chromosome has been rarely reported. It is characterized by a significant degree of phenotypic abnormalities including multiple congenital anomalies, short stature, and mental retardation. The external genitalia may be male or female, or ambiguous. We report what we believe is the first de novo Xp duplication detected at prenatal diagnosis. Cytogenetic analysis was performed on an amniotic fluid specimen from a 41 year-old female at 13.1 weeks gestation due to advanced maternal age. All cells showed a normal Y chromosome and an abnormal X chromosome with additional material on the short arm. This abnormality was determined to be a duplication of the short arm from band p21.2 to p22.3 by FISH using whole chromosome paint X probe, and probes specific for the steroid sulfatase (STS) gene, Kallmann (KAL) gene and Xp subtelomere (Vysis, Inc.). Maternal blood cytogenetic analysis revealed a normal karyotype. The fetal karyotype was therefore designated as: 46,Y,add(X)(p22.1)dn.ish dup(X)(p21.2p22.3)(wcpX+,Xpter+,STS++,KAL++). Duplications in Xp including the dosage sensitive sex reversal (DSS) region can cause male to female sex reversal. It has been shown that the gene NR0B1 (DAX-1) located at Xp21.2p21.3 is a dosage sensitive gene, preventing the formation of male reproductive tissues when duplicated. This gene encodes a protein containing a DNA-binding domain which functions as an anti-testis gene by acting antagonistically to SRY. The pregnancy is continuing and clinical outcome will be provided.

Overexpression of Wwp1 causes left ventricular hypertrophy and sudden death in a novel transgenic mouse model. *L. E. Matesic[1], J. B. Lea[1], W. Basheer[1], A. J. Dupuy[2], D. A. Swing[2], N. G. Copeland[2], N. A. Jenkins[2], R. L. Price[3]* 1) Department of Biological Sciences, University of South Carolina, Columbia, SC; 2) Mouse Cancer Genetics Program, National Cancer Institute at Frederick, Frederick, MD; 3) Department of Cell and Developmental Biology, University of South Carolina School of Medicine, Columbia, SC.

Familial hypertrophic cardiomyopathy (FHC) is the most common genetic myocardial disease and is the leading cause of sudden cardiac death in athletes and young people. FHC is characterized by left ventricular hypertrophy in the absence of hemodynamic stress with areas of myocyte disarray in the myocardium. Although most known FHC-causing mutations map to genes that encode sarcomeric proteins, recent findings indicate that the molecular mechanism underlying some of the more common mutations may be an impairment of the ubiquitin proteasome system (UPS). In order to test the contributions of the UPS to FHC, we created an inducible transgenic mouse that globally overexpresses the ubiquitin ligase Wwp1. Overexpression of Wwp1 contributes to epithelial hyperplasia and transformation in breast and prostate cancer but has not been previously associated with FHC. Mice globally overexpressing Wwp1 died very suddenly at 7-12 weeks of age. This phenotype was 100% penetrant in two independent transgenic lines. Examination of Wwp1 overexpressors by histology, by echocardiography, and by confocal measurement revealed concentric left ventricular hypertrophy. Further, transgenic animals displayed a significant increase in heart weight to body weight ratio, and myocyte disarray with disruption of intercalated discs was noted by confocal microscopy. The mammalian heart is known to undergo postnatal remodeling which establishes the intercalated disc structure and redistributes gap junctions. These changes underlie alterations in the rate and anisotropy of conduction during postnatal maturation. Our results are consistent with the assertion that hyperactivation of the UPS interferes with this remodeling, leading to electrical conduction defects and death, and suggest that the UPS may be a novel therapeutic target for FHC.

Empirical evaluation of genome-wide allelotyping on pooled DNA samples. *C. W. K. Chiang[1,2], Z. K. Z. Gajdos[1,2], F. G. Kuruvilla[2], J. M. Korn[2], R. Cooper[3], X. Zhu[4], H. N. Lyon[1,2], J. N. Hirschhorn[1,2]* 1) Harvard Med School & Childrens Hospital, Boston MA; 2) Broad Inst, Cambridge MA; 3) Loyola Univ Chicago, Chicago IL; 4) Case Western Reserve Univ, Cleveland OH.

Genome-wide association studies (GWAS) have recently proven successful in identifying common variants associated with complex diseases and quantitative traits, such as diabetes and height. A drawback to such studies is that a well-powered design is costly if each subject is genotyped individually, making GWAS less feasible for very large studies. The relatively high cost of custom genotyping prohibits extensive follow-up of potential signals using a staged design. GWAS conducted by allelotyping pooled DNA samples has been proposed as a cost-efficient alternative screening method to prioritize potential associations. However, a pooled GWAS design has not yet been evaluated on the Affymetrix 6.0 array, and few studies have compared results from pooled cases and controls to that from individual genotype data for the same individuals. We report the construction of five DNA pools composed of mixtures of the HapMap YRI and CEU individuals in varying proportions. DNA pools were made in triplicate, and allelotyped using the Affymetrix 6.0 array; for association testing, different pools designated cases and controls. Normalized polar transformations of SNP intensity readings were used to estimate allele frequencies. Informed by the actual allele frequencies from individual genotype data, we established quality control filters based on the radius of the average pool intensity in polar coordinates, separation of genotype clusters, and the spread in allele frequency estimate errors across pools. We also corrected for pool-specific experimental variation to avoid inflation of the ^{2} statistics. After QC filtering, we assayed approximately 450K autosomal SNPs. Among SNPs with a corrected $P<10^{-7}$, the QC filters lowered the proportion of false positives (defined as SNPs with an expected P value of greater than 10^{-4}) from 0.89 to 0.54. We have also allelotyped four pools of obese and lean African Americans for which we have the individual genotype data to further validate the effectiveness of our filters.

Blood pressure-related aminopeptidase genes show putative genetic association with preeclampsia. *M. P. Johnson[1], T. D. Dyer[1], K. A. Freed[1], C. E. East[2], J. Blangero[1], S. P. Brennecke[2], E. K. Moses[1]* 1) Deptartment of Genetics, Southwest Foundation for Biomedical Research, San Antonio, TX U.S.A; 2) Deptartment of Perinatal Medicine, Royal Women's Hospital & Deptartment of Obstetrics & Gynaecology, University of Melbourne, Melbourne, Australia.

We have previously reported strong evidence of a preeclampsia susceptibility locus on chromosome 5q in a cohort of Australian and New Zealand preeclampsia families. The resolution of this 5q locus posits the next challenge of positional candidate gene selection for more in depth genetic and (potential) functional examination. Initial molecular interrogation has to date incorporated the selection of publically available exon-centric and putative regulatory SNPs for evidence of association/linkage disequilibrium. Strong genetic associations with SNPs in two aminopeptidase genes, ERAP1 (rs3734016; $p=9\times10^{-3}$) and ERAP2 (rs2549782; $p=4\times10^{-3}$), were identified. The Endoplasmic Reticulum Aminopeptidase 1 and 2 genes encode enzymes belonging to an oxytocinase subfamily of M1 zinc-containing aminopeptidases. Contiguously located on chromosome 5q15 these genes are proposed to play an important role in the metabolism of peptides potentially involved in blood pressure and in the pathogenesis of essential hypertension. These are plausible candidates in the context that preeclampsia is characterized in part by new onset hypertension during pregnancy and that preeclamptic pregnancies are known to elevate the risk of women developing later life cardiovascular disease. These positional candidate genes are therefore of great interest in our current efforts to unravel the allelic architecture of preeclampsia.

Detection of population substructure among Jews and a north/south gradient within Ashkenazi Jews using 32 STR markers. *J. Listman[1], D. Hasin[2], H. R. Kranzler[3], A. Frisch[4], A. Weizman[4], E. Aharonovich[2], R. T. Malison[5], A. Mutirangura[6], A. Sughondhabirom[6], J. Gelernter[5]* 1) Dept Anthropology, New York U, New York, NY; 2) Dept Psychiatry, Columbia U College Physicians & Surgeons, NY, NY; 3) Dept Psychiatry, U CT Sch Med, Farmington, CT; 4) Sackler Faculty of Med, Tel Aviv U, Tel Aviv, Israel; 5) Dept Psychiatry Yale U Sch Med, New Haven, CT; 6) Chulalongkorn Faculty of Med, Bangkok, Thailand.

Understanding and detecting population substructure are critical issues. Using 32 autosomal STR markers and the program STRUCTURE we demonstrated differentiation between Ashkenazi (AJ) (N=135) and Sephardic (SJ) (N=226) Jewish populations in the form of Northern and Southern European genetic components (AJ north 73%, south 22%, SJ north 32%, south 61%) and a significant relationship between latitude of grandparental country of origin (GCO) and percent north/south genetic component in AJ. Notably, we revealed substructure among Jews (and among European Americans (EA)) using a small STR panel, only when additional samples representing major continental populations (African American, EA, Asian) were included in analyses. Further, negative R_{IS} (-0.035) indicates recent admixture in individuals with both SJ and AJ parents (N=38). R_{IS} is a measure of inbreeding adapted from F_{IS} for STR markers. Negative R_{IS} indicates allelic variation within individuals greater than expected under random mating, i.e., excess heterozygosity due to outbreeding. Although geographic patterns are seen in the average north/south percent assignment values between groups as defined by AJ or SJ, grandparental world region of origin, or GCO, within each group there is high variability among individual assignment values. Thus, even based on data from a small marker set, AJ is not a homogeneous population. The north/south gradient in AJ may be a reflection of the pre-existing north/south gradient in European host populations (recently shown in other studies using large numbers of SNPs) with which Jews admixed slowly. We also demonstrate the utility of including purported parental populations when attempting to detect population substructure within closely related populations.

RAAS and Inflammation Factors Regulate Telomerase Expression in the Mouse Cortical Collecting Duct Cells.
Y. Dong, K. Li, DH. Guo, J. Thomas, H. Zhu Georgia Prevention Inst, Medical Col Georgia, Augusta, GA.

Background: Telomerase complex consisting of a reverse transcriptase catalytic subunit (TERT) and an RNA component (TERC) is responsible for telomeric maintenance and extension. Oxidative stress, inflammation and renin-angiotensin-aldosterone (RAAS) system have been shown to increase the rate of telomere attrition in vitro and in vivo. However, their effects on renal distal tubular cells are not well understood. Aims: To elucidate the effects of markers of RAAS and inflammation on the regulation of telomerase expression in mouse cortical collecting duct cells. Methods: M-1 cortical collecting duct cells were cultured and serum-deprived for 24 hours, and cells then were treated with aldosterone (1M), angiotensin II (10M), IL-6 (100ng/mL), TGF-1 (20ng/mL) and TNF- (100ng/mL) for 24 hours. Total RNA was extracted, and real-time quantitative RT-PCR was conducted. The amount of TERT and TERC expressed were normalized to the endogenous control -actin. The relative quantification was determined by comparative CT method. Results: Both aldosterone and IL-6 decreased TERT expression by 38 5%, p=0.004 and 20 4%, p=0.008 respectively. Angiotensin II decreased TERT expression by 24 5%, p=0.009, whereas increased TERC expression by 73 11%, p=0.01. Both TERT and TERC expression did not change after 24 hour of treatment by TGF-1, however, TERT expression was reduced by 23 6%, p=0.018 after 12-hour treatment. TNF- slightly increased TERC expression by 18 4%, p=0.015. Conclusion: This is the first study to show that aldosterone, angiotensin II, IL-6 and TGF-1 inhibit mouse cortical collecting duct cell TERT expression. On the other hand, angiotensin II and TNF- stimulate mouse cortical collecting duct cell TERC expression. The mechanisms are not clear and warrant further investigation.

Using the mouse model to confirm genes identified by genome-wide association studies for the complex trait HDL cholesterol. *M. S. Leduc[1], R. Korstanje[1], M. Orho-Melander[2], S. Kathiresan[3], B. Paigen[1]* 1) The Jackson Laboratory, Bar Harbor, ME; 2) University Hospital Malmo, Lund University, Malmo, Sweden; 3) Massachusetts General Hospital, Boston, MA.

Plasma high density lipoprotein cholesterol (HDL) level is a complex trait associated with risk of cardiovascular events. Recently, a human genome wide association study (GWAS) involving 8,816 individuals (Kathiresan et al., Nat. Genet., 2008) identified putative new loci determining plasma HDL. However, due to the multiplicity of hypotheses tested in GWAS, validation of these putative loci is required. Traditionally, validation occurs through replication by association analysis in other human populations. However, we suggest that the mouse model may also by used to provide supportive evidence if the significant results is in a genomic region homologous to quantitative trait loci (QTL) in the mouse. We previously mapped all known mouse HDL QTLs on the mouse genome (Wang and Paigen, Circ. Res., 2005). From the GWAS, we reduced the 3382 significant SNPs (P<10-3) to 1121 bins based on linkage disequilibrium and selected 171 bins containing at least one highly significant SNP (P<10-4). The 171 bins from this human study were positioned to homologous regions in the mouse, leading to 98 genomic loci in the mouse genome. A total of 85% of these loci lay within 20 Mb of a HDL QTL peak in the mouse. Some of these confirmed major key players in HDL metabolism, already known from other human and mouse studies (*Lpl, Lcat, Lipc, Abca1, Lipg*). By comparing the strains that were used for the QTL studies using our bioinformatic tool, we were able to confirm several putative genes from the GWAS study as mouse QTL gene (*Tshr, Pcsk5, Galnt2, Ppara*). Evidences included haplotype differences, amino acid changes in the coding region, significant expression changes between the strains, and other bioinformatic tools. Finding that the same gene was responsible for the QTL in the mouse therefore adds confirmatory evidence to the human GWAS gene. We conclude that mouse-human genomic comparison is a powerful tool to identify and validate complex trait genes and will help decipher results from human GWAS.

Analysis of Kalirin Polymorphisms with Cardiovascular Risk, Type 2 Diabetes, Metabolic Syndrome in the Diabetes Heart Study. *M. E. Rudock, J. T. Ziegler, A. B. Lehtinen, B. I. Freedman, J. J. Carr, C. D. Langefeld, D. W. Bowden* Wake Forest Univ Sch Med, Winston-Salem, NC.

Coronary artery disease (CAD) is the most common cause of death in type 2 diabetes (T2D) and all manifestations of cardiovascular disease are substantially more common in diabetic than in non-diabetic patients. Following a genome wide linkage scan for CAD in the GENECARD population, peak wide mapping of the 3q13 region identified KALRN as a candidate gene for CAD (Wang et al., Am.J.Hum.Genet., 2007). KALRN is thought to play a role in the development of CVD and T2DM via binding to RAC1. The KALRN locus lies under a linkage peak for prevalent CVD previously identified in a genome wide scan of the Diabetes Heart Study, DHS (Bowden et al., Diabetes, 2006). In an attempt to replicate this association, we chose to evaluate the relationship of polymorphisms in KALRN with cardiovascular phenotypes of the DHS. 28 SNPs in KALRN were genotyped in 977 Caucasian siblings from 369 DHS families, as well as 724 controls. Primary discrete traits were: +/- T2D, +/- metabolic syndrome (MS), +/- prevalent CVD (self-reported history of clinical CVD) and +/- combined-phenotype (+T2D, +MS, +CVD, and +coronary calcified plaque; CCP), which were analyzed for association using GEE1. The strongest evidence of association among discrete traits was seen with rs4234218, which was associated with T2D, MS and the combined-phenotype with p-values of 0.008 (OR=0.863), 0.006 (OR=0.825), and 0.014 (OR=0.889), under the dominant genetic model. Tests for single SNP association with quantitative traits relating to cardiovascular disease, including C-reactive protein (CRP), were performed using SOLAR (age and gender adjusted). The strongest evidence of association within the quantitative traits was with 6 SNPs and CRP (p=0.003 to 0.04 under the dominant model). In order to examine our data for evidence that smoking modulates the genetic effects of KALRN, we stratified our population by smoking status. The association among quantitative traits is more pronounced (p=0.0001 to 0.049) in smokers rather than the non-smokers. We conclude that KALRN variants are associated with presence of T2D, MS and CVD risk factors.

Gene expression in muscle degeneration and regeneration pathways, in different mice models for muscular dystrophies. *P. C. G. Onofre-Oliveira, P. C. M. Martins, V. L. Ferreira, D. Ayub-Guerrieri, M. Vainzof* Human Genome Research Center, University of São Paulo, São Paulo, São Paulo, Brazil.

Muscular dystrophies (MD) are characterized by progressive and irreversible weakness. Muscle degradation is the consequence of disequilibrium between the degeneration and regeneration processes. In the dystrophic process, some cytokines such as TGF-b1, when in high concentrations, can activate the production of collagen, promoting tissue fibrosis. Regeneration is controlled mainly by the myogenic factors MyoD and Myf5 (activation and proliferation of the quiescent mesenchimal precursors), and Myf6 and Myogenin (differentiation and fusion of the activated myoblasts into the affected muscle). Several mice models for genetic MD are recognized, presenting variable histopathological and clinical findings. Mice MDX, SJL/J, and dy2J/dy2J are models for Duchenne, Limb-girdle-2B and congenital-1A MDs, respectively. Using Real-Time PCR, we have been studying the differential expression of genes TGF-b1 and PCOL1a2 (Pro-collagen) from the degeneration pathway, and MyoD, Myf5 and Myf6 from the regeneration pathway, in these adult mice models. Our preliminary results show some residual expression of TGF-b1 and PCOL1a2 in normal muscles, which are not sufficient to activate the regeneration pathway, as observed through the very low expression of MyoD and Myf5 genes. As expected, the more severely degenerated dy2J/dy2J muscles showed the highest levels of PCOL1a2 and TGF-b1. The regeneration process, however, was also very active, in spite of its significant clinical weakness. The mildly affected SJL/J mouse showed a very low expression of regeneration genes with some prevalence of Myf5. On the other hand, MDX mouse, whose muscle histology presents the highest regeneration pattern, showed similar levels of expression for all studied genes. Our results suggest there is no direct correlation between the muscle histological degeneration/regeneration patterns, and the expression of genes of the degeneration/regeneration pathways. More tests are being conducted on animals of different ages to better clarify these mechanisms. FAPESP-CEPID, CNPq, ABDIM-BR.

Combining two genome wide association scans for seven smoking related phenotypes replicates published associations in the CHRNA3/5 region. *F. Gu[1], A. Bergen[2], N. Chatterjee[3], J. Sheng-Shih[5], K. Yu[3], M. Yeager[4], D. H. Hunter[1], G. Thomas[4], K. Jacobs[4], M. T. Landi[3], S. Chanock[4], J. Chen[1], R. Ziegler[3], N. Caporaso[3], P. Kraft[1]* 1) Program in Molecular and Genetic Epidemiology, Harvard School of Public Health, Boston, MA; 2) Molecular Genetics Program, SRI International; 3) Division of Cancer Epidemiology and Genetics, National Cancer Institute; 4) Core Genotyping Facility, National Cancer Institute; 5) Johns Hopkins University.

Smoking is a risk factor for more than two dozen diseases and conditions and a leading contributor to mortality worldwide. We performed genome-wide association scans for seven smoking behavior phenotypes using data on 1,144 breast cancer cases and 1,138 controls from the Nurses' Health Study and 1,065 prostate cancer cases and 995 controls from the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial. We tested the association between these phenotypes and over 518,350 SNPs on the Illumina HumanHap 550k platform that passed quality control and had minor allele frequency >1% in both studies. We combined study-specific results using weighted Z scores. Although no SNPs achieved genome-wide significance ($p<10-7$) for any phenotype, we replicated published associations between SNPs in the CHRNA3/CHRNA5 region and cigarettes per day (CPD). We also found evidence that variation in the candidate genes MAOA, TRPV1, and FOSB was associated with CPD (gene-level $p<0.01$). Our study provides further evidence that SNPs in the CHRNA3/CHRNA5 region are associated with smoking behavior, and suggests several other regions for further study.

Identification and functional analysis of schizophrenia risk variants at the DTNBP1 locus. *N. Williams, L. Carroll, A. Gerrish, L. Elliston, G. Kirov, M. Owen, M. O'Donovan* Psychological Medicine, Cardiff University, Cardiff, United Kingdom.

A large volume of genetic association data from multiple populations now supports the locus encoding Dysbindin (DTNBP1) as a schizophrenia susceptibility gene. The source of the genetic association at DTNBP1 is equivocal, however studies to date show altered protein and mRNA levels in schizophrenic CNS. Furthermore, there is evidence to suggest that cis acting variation alters Dysbindin mRNA levels in post-mortem neural tissue and is correlated with a replicated schizophrenia at-risk haplotype. To identify the functional variation causing the association at DTNBP1 we have performed resequencing and high-density association mapping in case-control and familial schizophrenia samples from Europe. Refining the association signal in both samples has yielded intriguing evidence for allelic heterogeneity at this locus. Associated variants in both samples have been analysed to see whether they account for the changes in mRNA levels observed in post-mortem brain by both allelic expression analysis and also via luciferase reporter assay.

Determining the Genetic Architecture of Central Cornea Thickness (CCT) with GWAS using DNA Pooling. *A. B. Ekici[1], J. Etschel[1], S. Uebe[1], F. Pasutto[1], F. E. Kruse[2], C. Y. Mardin[2], A. Reis[1]* 1) Institute of Human Genetics, Friedrich-Alexander-University Erlangen-Nuremberg, Erlangen, Germany; 2) Department of Ophthalmology, Friedrich-Alexander-University Erlangen-Nuremberg, Erlangen, Germany.

Purpose: Our aim was to identify quantitative trait loci which influence central cornea thickness (CCT). Here, cornea thickness refers to the average of both eyes, since correlation of CCT in both eyes had been determined previously. CCT values from 600 individuals of German descent showed a normal distribution. **Methods:** We performed a genome-wide association analysis using SNP-MaP (SNP Microarray and DNA pooling) with 500,568 SNP Affymetrix arrays. We pooled DNA of 36 individuals from either extreme of the normal distribution in 3 replicates (n=12). Array analysis of pooled DNAs was done with a modified version of GenePool (Pearson et al., AJHG 2007; 80(1):126-39). For graphical visualization of GenePool data, we developed the software GPGraphics to facilitate analysis. **Results:** We correlated the difference in mean RAS values of both pools (RASmean) with GenePool ranks for each SNP. Distinct differences were only present among the first 2,000 SNPs. Accordingly, we defined clusters with at least 3 SNPs with a rank <1,000 as associated loci. Our analysis identified 40 loci with marked RASmean values as indication of differential allele frequencies between both extreme quantiles. We also reproduced the association by comparing the extreme groups with pools of population based controls. 34 of these SNP loci showed strong LD in HapMap data and 26 pointed to genes. We then verified pooling data at single genotype level for two selected loci using TaqMan assays in the complete cohort. htSNPs selected to contain at least 1 SNP with a rank <2,000 confirmed significant allele frequency differences (p=0.001), even after permutation correction (p=0.011). **Conclusion:** Our data show that hybridization intensities of pooled DNA correlate well with individual genotyping results. Thus DNA pooling is a useful strategy for GWAS, although loci with strong LD are preferentially detected on those arrays. Furthermore, the genetic architecture of CCT is extremely complex with at least 40 loci influencing this trait.

The importance of developing comprehensive mutation analysis in a clinical diagnostic setting. *L. Bean, B. Coffee, C. Alexander, E. Chin, M. Hegde* Dept Human Genetics, Emory Univ, Atlanta, GA.

Full gene sequence analysis to detect mutations in disease genes is common place in clinical diagnostic laboratories. Current technology allows rapid development and implementation of sequencing assays. Since mutation identification is of paramount importance for diagnosis confirmation, genetic counseling, risk assessment and carrier screening, efforts must be made to perform comprehensive mutation analysis. Although methods such as Southern blotting, quantitative PCR methods and MLPA are used in clinical laboratories, they can be time consuming, laborious, insensitive and even inaccurate. As we enter the era of rare disorder testing the true mutation spectrum for many rare disorders is not known. Comparative Genomic Hybridization (CGH), which has undergone rapid development in the last few years, offers is a powerful alternative to the current methods used for detecting large deletions and duplications. We have developed a single high resolution array to detect single and multi-exon deletion or duplication mutations in a large set of genes. Using this technology, we have detected novel copy number changes within several genes including, GALC (Krabbe disease) and BCKDHB (MSUD). In addition to a common 30kb GALC gene deletion we have identified 3 novel deletions and 1 novel duplication in the GALC gene. Molecular diagnostics for MSUD present several unique diagnostic challenges. Mutations in three unlinked genes (BCKDHA, BCKDHB, and DBT) in which few causative mutations have been reported cause an identical MSUD phenotype. Identification of missense changes can leave substantial doubt as to their causative nature. We have identified one novel duplication and one novel deletion within the BCKDHB gene in two patients with 1 mutation identified by sequencing. In these cases, carrier testing for family members at risk for carrying the mutations and prenatal testing could be offered following identification of a deletion or duplication. Despite the maturity of sequencing and CGH, an estimated 1-2% of mutations located deep within intronic or promoter regions cannot be identified. Future work will focus on accomplishing truly comprehensive mutation analysis.

A biochemical link between Gaucher and Parkinson's diseases suggests a potential approach to treating synucleinopathies. *S. Clark[1], Y. Sun[2], Y. H. Xu[2], G. A. Grabowski[2], B. A. Wustman[1]* 1) Amicus Therapeutics, Cranbury, NJ; 2) Children's Hospital Research Foundation, Cincinnati, OH.

Mutations in the GBA gene that encodes the lysosomal enzyme glucocerebrosidase (GCase) are a risk factor for Parkinsons disease and Dementia with Lewy Bodies. Homozygous mutations in GBA lead to Gaucher disease due to reduced enzyme activity and accumulation of glucosylceramide (GlcCer), and a 2-fold increase in -synuclein is sufficient for Parkinson's. We hypothesized that the decreased GCase activity of carriers and ensuing accumulation of GlcCer may interfere with the normal degradation of synuclein, increasing synuclein levels. We examined a Gaucher mouse model that combines a GBA mutation with reduced expression from the Prosaposin locus. Prosaposin is processed into Saposins including the GCase-activating protein Saposin C. Combining the GBA mutation with reduced Saposin expression results in the accumulation of GlcCer in the brain and viscera. These mice accumulate -synuclein in the cortex and hippocampus. Mice harboring the GBA mutation, but wild-type expression of Prosaposin, do not accumulate GlcCer or -synuclein. This suggests accumulation of -synuclein is correlated with increased GlcCer or a related sphingolipid. Exploring the GCase--synuclein relationship further, we utilized a mouse model moderately overproducing wild-type human -synuclein in the hippocampus, cortex, and olfactory bulb ([PDGF]pr-hSNCA). Unlike the Gaucher mouse model, these mice express endogenous, wild-type GCase. The pharmacological chaperone, AT2101 (isofagomine), has been shown to increase GCase activity in mice and humans. Treatment of these mice with AT2101 prevented the age-dependent accumulation of human -synuclein in neurons in the hippocampus and to a lesser extent in the cortex. We found that AT2101 also prevented an age-dependent increase in Campbell-Switzer-positive aggregates in the hippocampus. This suggests that increase of GCase activity by AT2101 reduces the steady-state level of -synuclein, leading to reduced accumulation of -synuclein aggregates in the brain.

Maternal Age and Risk for Trisomy 21 Assessed by the Origin of Chromosome Nondisjunction: A Report from the Atlanta and National Down Syndrome Projects. *S. Sherman[1], E. G. Allen[1], C. Druschel[2], C. A. Hobbs[3], P. A. Romitti[4], M. H. Royle[5], C. P. Torfs[6], S. B. Freeman[1]* 1) Dept Human Gen, Emory Univ, Atlanta, GA; 2) New York State Dept of Health, Troy, NY; 3) Univ of Arkansas for Medical Sciences, Little Rock. AR; 4) The Univ of Iowa, Iowa City, IA; 5) New Jersey Dept of Health and Senior Services, Trenton, NJ; 6) Public Health Inst, Birth Defects Studies, Emeryville, CA.

The purpose of our study was to examine the association between maternal age and chromosome 21 nondisjunction (NDJ) by the origin of the meiotic error. We analyzed data from two population-based, case-control studies: Atlanta Down Syndrome Project (1989-1999) and National Down Syndrome Project (2001-2004). Cases were live born infants with trisomy 21 or mosaic trisomy 21 and controls were live born infants without trisomy 21 delivered in the same geographical regions. Of the eligible 1881 cases and 2293 controls, we enrolled 1215 case and 1375 control families (64.6% and 60.0% participation, respectively). Chromosome 21 DNA markers were used to classify the NDJ error. There were four primary findings from this study. First, the significant association between advanced maternal age and chromosome 21 NDJ was restricted to meiotic errors in the egg; the association was not observed in sperm or in mitotic errors. Second, advanced maternal age was significantly associated with errors occurring in both stages of meiosis. For example, compared to mothers of controls, mothers of infants with trisomy 21 due to meiosis I (MI) NDJ were 8.5 times more likely to be 40 years old than 20-24 years old at the birth of the index case (95% CI= 5.6-12.9). Where NDJ occurred in meiosis II (MII), mothers were 15.1 times more likely to be 40 years (95% CI=8.4-27.3). Third, the ratio of MI to MII errors differed by maternal age. The ratio was lower among women 19 years of age and those 40 years (2.1, 2.3, respectively) and higher in the middle age group (3.6). Lastly, there was no evidence found for a grandmaternal age effect for the risk for maternal NDJ. This study emphasizes the complex association between advanced maternal age and NDJ of chromosome 21 during oogenesis.

Association analysis of the interleukin 17A (*IL17A*) gene in Rheumatoid Arthritis patients from Norway and New Zealand. *G. B. N. Nordang[1], M. K. Viken[1], J. E. Hollis-Moffatt[2], T. R. Merriman[2], O. T. Forre[3], K. Helgetveit[4], T. K. Kvien[5], B. A. Lie[1]* 1) Institute of Immunology, Rikshospitalet University Hospital, Oslo, Norway; 2) Department of Biochemistry, University of Otago, Dunedin, New Zealand; 3) Department of Rheumatology, Rikshospitalet University Hospital, Oslo, Norway; 4) Martina Hansens Hospital, Sandvika, Norway; 5) Department of Rheumatology, Diakonhjemmet Hospital, Oslo, Norway.

Objective: Elevated levels of interleukin 17A (IL-17A) have been detected in the inflamed synovium of rheumatoid arthritis (RA) patients, and murine arthritis models that are *IL17A* deficient demonstrate reduced inflammation. A possible indirect effect during osteoclatsogenesis has been revealed by IL-17A-mediated induction of receptor activator of NF-B ligand (RANKL) on osteoblastic cells (Sato K et al. 2006). Based on the likely role of IL-17A in inflammation and bone destruction in RA, the aim of this study was to investigate *IL17A* as a candidate gene for RA. **Method:** Five single nucleotide polymorphisms (SNP) were selected to tag the genetic variability of the IL17A region. Genotyping was performed by TaqMan technology on 950 RA cases and 933 random controls from Norway. In addition, 652 RA patients and 562 controls from New Zealand were used as a replication data set. **Results:** We found a weakly significant association between RA and the promoter SNP rs2275913 (OR=1.17; 95% CI [1.02 - 1.34]; *P*=0.02) in the Norwegian population. This association was not replicated in the RA cohort from New Zealand (OR=1.01; 95% CI [0.93 - 1.10]; *P*=0.8). However, combining the two data sets demonstrates a weak association (OR= 1.20; 95% CI [1.03 - 1.39]; *P*=0.02). **Conclusion:** Modest evidence of an association with *IL17A* in Norwegian RA patients was observed. Although, our findings were not replicated in an independent RA cohort from New Zealand, a significant common risk estimate indicated that *IL17A* warrants further investigation in RA before any definite conclusions can be drawn.

A small in-frame deletion in the seventh immunoglobulin-like repeat of filamin C is a cause of myofibrillar myopathy. *A. Shatunov[1], M. Olivé[2], Z. Odgerel[1], C. Stadelmann-Nessler[3], K. Irlbacher[4], M. Bayarsaikhan[5], H.-S. Lee[1], I. Ferrer[2], M. C. Dalakas[6], N. Sambuughin[5], H. H. Goebel[7], L. G. Goldfarb[1]* 1) National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland, U.S.A; 2) Institut de Neuropatologia, Ciutat Sanitària i Universitària de Bellvitge, Hospitalet de Llobregat, Barcelona, Spain; 3) Universitätsmedizin der Georg-August-Universität Göttingen, 37075 Göttingen, Germany; 4) Charité - Universitätsmedizin Berlin, Germany; 5) Uniformed Services University of the Health Sciences, Bethesda, Maryland, U.S.A; 6) Thomas Jefferson University, Philadelphia, PA, U.S.A; 7) Mainz University Medical Center, Mainz, Germany.

We studied an International cohort of 39 families with myopathologically confirmed myofibrillar myopathies, in which DES, CRYAB, MYOT, and ZASP mutations have been excluded. In an unrelated German family a 12-nucleotide deletion (c.2997_3008del) in FLNC resulting in a predicted in-frame 4-residue deletion (p.Val930_Thr933del) in the 7th repeat of filamin C was identified. Both affected family members, mother and daughter, carried the mutation. Size-fragment analysis of FLNC exon 18 in 241 unrelated individuals of European ancestry failed to uncover defects in exon 18, suggesting that p.V930_T933del was the candidate mutation associated with myofibrillar myopathy in this family. Ultrastructural examination revealed major myofibrillar abnormalities, including accumulation of debris of Z-disc origin, granular and filamentous material and the presence of a large number of nemaline rods. In addition, there were tubulofilamentous profiles and large autophagic vacuoles containing myelin-like figures and cellular debris. V930_T933del deletion in FLNC can cause damage in the cytoskeletal architecture and most likely is a structural basis for deficient flexibility of actin networks. Molecular analysis of the novel filamin C mutation adds to the characterization of the diverse forms of myofibrillar myopathies and permits a more precise molecular diagnosis.

Genetic association with retinopathy in a large cohort of American families with type 1 diabetes. *C. Monti[1,3], R. Montross[2], B. Corso[3], J. Lonsdale[2], C. Montmoli[3], D. Greenberg[1]* 1) Division of Statistical Genetics, Department of Biostatistics, Columbia University, New York, NY; 2) Genetics Division (HBDI), National Disease Research Interchange, Philadelphia, PA; 3) Section of Medical Statistics and Epidemiology, Department of Health Sciences, University of Pavia, Pavia, Italy.

We conducted a case-control analysis to test for association of diabetic retinopathy with single nucleotide polymorphisms (SNPs) found in type 1 diabetes (T1D) candidate loci. All subjects had T1D; cases had the complication of retinopathy, controls did not. Phenotypic data came from the Human Biological Data Interchange (HBDI) family collection of the National Disease Research Interchange (Phila., PA). SNP data was from the Type 1 Diabetes Genetics Consortium, sponsored by NIDDK, NIAID, NHGRI, NICHD and JDRFI. Of the 6000 available SNPs, we focused on SNPs known to be in T1D-related genes. The phenotype (presence or absence of retinopathy) was rigorously assessed. Cases had clearly-diagnosed retinopathy. Controls had T1D for 20 years or more without retinopathy and had no first-degree relative family history of retinopathy. We compared the frequency of alleles in 118 retinopathy T1D probands (cases) with the frequencies in 143 non-retinopathy T1D probands (controls). The SNPs that yielded strong association evidence for retinopathy susceptibility ($p<0.001$, $OR > 1.7$) were: rs540652 (NOSTRIN gene), rs1991537 (ADAM23 gene), and rs1483457 (LOC389676 gene). Two SNPs showed a significant ($p<0.001$, $OR <0.5$) protective effect: rs9740 (CASR gene) and rs3740892 (PKNOX2 gene). These results provide strong preliminary evidence for genetic association with diabetic retinopathy. Our results suggest that genes predisposing to T1D may also be involved in increased risk for complications, although some may provide protective alleles.

DSS1, Abraxas and RAP80: an analysis of three genes coding for BRCA1 or BRCA2 interacting proteins in hereditary breast cancer families. *D. J. Novak[1, 2], N. Sabbaghian[2], R. Kyle[2], P. Maillet[3], P. O. Chappuis[3], M. Tischkowitz[1,2], W. D. Foulkes[1,2]* 1) Depts of Medicine and Human Genetics, McGill University, Montreal , Canada; 2) Program in Cancer Genetics, McGill University, Montreal , Canada; 3) Service of Oncology and Med Gen, Geneva University Hospitals, Geneva, Switzerland.

Background: Germ-line mutations in the two major breast cancer (BrCa) susceptibility genes, BRCA1/2, in addition to minor susceptibility genes such as CHEK2, P53, ATM, PTEN, BRIP1 and PALB2 currently account for 30-50% of the familial BrCa risk. Most of these genes encode proteins implicated in the preservation of genomic integrity, interacting either directly or indirectly with one of the BRCA genes. Three genes with similar functional interactions are DSS1, RAP80 and Abraxas. DSS1 is known to interact with BRCA2, and is implicated in BRCA2 DNA repair of DSBs, in addition to DNA foci formation of RAD51. Abraxas has been shown to directly interact with BRCA1, allowing for the indirect interaction between BRCA1 and RAP80. Together, the BRCA1-Abraxas-RAP80 complex is required for loading BRCA1 to sites of DNA damage and its associated response. Mutations in any of these three genes may account for unattributed familial BrCa risk.

Methods: One hundred and forty seven BRCA1/2 negative women with BrCa, selected for a strong family history of multiple BrCa cases, were sequenced for variants within the 3 exons of DSS1. Sequencing data was analyzed as an entire set as well as according to ethnic background. Similarly, ninety-five women with BrCa were sequenced for variants within the 14 exons of RAP80 and 9 exons of Abraxas.

Results: In DSS1, one silent variant was identified. In Abraxas, we have identified nine intronic, two untranslated and two previously identified missense variants. Finally, seven intronic, three silent, three known and one novel missense, in addition to three variants in the UTRs of RAP80, have been identified.

Conclusions: Overall, it appears unlikely that highly penetrant alleles exist in either of the DSS1, RAP80 or Abraxas genes.

Towards protein expression quantitative trait loci (peQTL): Initial analysis of variability in protein expression levels and development of a 2D gel spot-map for human lymphoblastoid cells. *M. K. Bunger[1], M. D. Rowland[1], H. Pan[2]* 1) Biomarkers and Systems Biology Center, RTI International, Research Triangle Park, NC; 2) Research Computing Division, RTI International, Research Triangle Park, NC.

Population-based variability in protein expression patterns are often observed, but poorly understood. Recent literature describing population-based mRNA expression implies that variability in mRNA levels behaves as a heritable quantitative trait. Several QTL associated with mRNA expression levels have been mapped in both *cis* and *trans* relative to expressed genes using genome wide association methods in multiple organisms. However, little is known about the genetics driving protein expression and whether the same QTL will be associated with both protein expression and mRNA expression variability. We have performed a small pilot study using quantitative fluorescence difference gel electrophoresis (DIGE) on proteins from 24 lymphoblastoid cell lines (LCLs) derived from the CEPH; Utah collection (CEU). On average, 1500 spots were detected on each gel and 560 spots were detected on all gels. Expression levels were determined relative to a pooled internal standard and population variability of each protein was calculated. Population variance among these proteins ranged from 0.006 to 36.2 demonstrating that a large dynamic range of variation is detectable using DIGE. Regression analysis of expression variation with cell doubling time revealed only a minor contribution with R^2 values above 0.5 for only 5 proteins. Identification of proteins from spots from 3 unrelated experiments was carried out using high resolution MALDI MS/MS incorporating both peptide mass fingerprinting and MS/MS analysis searching against the latest human protein database from IPI. To date 283 proteins have been identified from at least one gel. Among the 283 proteins, 58 were represented by at least two spots. Multiple spots per protein often occur as a result of post-translational modifications (PTMs) which can shift both the pI and molecular weight of any given protein, resulting in spot trains. Thus, using DIGE allows for quantitative analysis of both proteins and their post-translational modifications independently.

Identification of Genetic Variants Associated with Capecitabine toxicity through Gene Expression. *A. Stark[1], B. E. McIlwee[2], E. O. Kistner[3], M. E. Dolan[2]* 1) Dept Human Gen, Univ Chicago, Chicago, IL. USA; 2) Section of Hematology/Oncology, Dept Medicine, Univ Chicago, Chicago, IL USA; 3) Dept Health Studies, Univ Chicago, Chicago, IL USA.

Capecitabine is a prodrug of 5-fluorouracil used to treat colorectal and breast cancer. Human variation in the degree of chemotherapeutic-induced toxicity and response can be based on a number of functional mechanisms. In an attempt to elucidate these mechanisms we investigated the relationship between baseline gene expression levels and capecitabine-induced cytotoxicity in HapMap lymphoblastoid cell lines derived from the Phase I populations: Yorubans from Ibadan, Nigeria, Japanese from Tokyo, Japan, Han Chinese from Beijing, China, and CEPH from Utah. Due to insufficient cytidine deaminase expression, we used an activated form of capecitabine (5-DFUR). Inter-ethnic differences in sensitivity to capecitabine were observed with the Yoruban population displaying the most sensitivity. Using the Affymetrix Gene Chip Human Exon 1.0 Array, we correlated the baseline gene expression with cytotoxicity measured in the Yoruban and CEPH cell lines. Using this genome-wide approach, we discovered unique and common genes correlating with cytotoxicity in the two populations. At a permissive p-value cutoff of $p < 0.005$, there are 196 unique CEPH genes and 31 unique Yoruban genes and 4 genes that overlap: WIG1, DMXL2, FMNL3, and TNFAIP2. Baseline expression for both WIG1 and FMNL3 are significantly associated with the same SNP on chromosome one (rs161110). The most significantly associated gene, found in the CEPH population, is hemochromatosis, HFE, ($p = 7.06$ x 10-6) a gene that encodes a transmembrane protein regulating transferrin receptor. HFE expression is associated with a SNP on chromosome five (rs10040947), a possible trans regulator ($p < 0.00001$). Genes with baseline expression correlated with cytotoxicity of capecitabine provide evidence for these candidate genes to be evaluated for regulatory architecture.

A novel locus for autosomal dominant retinitis pigmentosa (ADRP) maps to chromosome 7p15. *J. S. Friedman[1], M. Brooks[1], R. Khanna[2], E. H. Trager[2], K. E. Branham[2], V. Ponjavic[3], L. Gränse[3], G. R. Abecasis[4], S. Andréasson[3], A. Swaroop[1,2]* 1) NIH/NEI/NNRL, NIH, Bethesda, MD; 2) Department of Ophthalmology, University of Michigan, Ann Arbor MI; 3) Department of Ophthalmology, Lund University Hospital, Lund, Sweden; 4) Center for Statistical Genetics, Department of Biostatistics, University of Michigan, Ann Arbor, MI.

Retinitis Pigmentosa (RP) constitutes a group of genetic eye diseases in which the photoreceptor cells in the retina degenerate, leading to irreversible vision loss. To date, 17 genes have been identified to cause autosomal dominant retinitis pigmentosa (RetNet). We are studying a large Scandinavian family with a slower than average progression rate and apparent autosomal dominant pattern of inheritance. We performed linkage analysis using the data obtained by hybridiziation of affected individual DNAs to 250K gene chip SNP arrays from Affymetrix. SNP data was examined using standard methods (Pedstats, GRR etc), and linkage was performed using Merlin. We identified a peak lod score of 5.0 on chromosome 7p15. No mutations were identified in a previously-reported RP9 gene, Pim-1 kinase associated protein, at 7p14. We have initiated a screen of genes in the minimal region as determined through haplotype analysis. The identification of this ADRP gene will assist in the better understanding of the mechanisms underlying RP and could lead to targeted therapies to alleviate this disease.

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A novel mutation (Ser186Leu) in rhodopsin is associated with an autosomal dominant form of congenital stationary night blindness (adCSNB) in a Chinese family. *Y. Jiang[1], S. Hu[1], X. Liu[2], S.-H. Chen[3]* 1) Beijing Genomics Institute, Beijing, China; 2) School of Optometry and Ophthalmology, Wenzhou Medical college, Wenzhou, Zhejiang, China; 3) Department of Pediatrics, University of Washington, Seattle , WA98195.

The autosomal dominant form of CSNB is a group of rare eye disorders characterized by non-progressive deficiency of night vision under dim illumination with normal fundus, abnormal dark adaptation curves and severe attenuation of electroretinogram (ERG) a and b waves. Molecular studies of families with adCSNB have identified six missense mutations in 3 different genes. They are: G90D, A292E, T94I of RHO; H258D of PDE6B; and G38D and Q200E of GNAT1. We have studied a large Chinese adCSNB family with over 34 affected individuals in 11 generations. A novel mutation, Ser186Leu of RHO was found to associate with the adCSNB in this family. Peripheral blood samples were obtained from 15 affected individuals, 2 toddles of unknown phenotype and 12 unaffected family members. Genomic DNA was isolated from leucocytes. Six segments of the RHO gene were amplified by a standard PCR method. Sequencing was performed in Beijing using an ABI prim 3730x1 Sequencer. Using Polyphred (Phred/Phrap/Consed software package), a mutation was identified in each of 15 affected family members and 2 toddles but not in the 12 unaffected individuals of the family or in 100 controls. The phenotype of adCSNB is co-segregating with the heterozygotes in the family. The mutation is a C to T missense mutation in codon 186.2 (TCG) of the RHO gene resulting in Serine (TCG) to Leucine (TTG) change at amino acid 186 of rhodopsin protein. The affected individuals and the 2 toddles are heterozygous (Serine/Leucine) for the locus, while the unaffected individuals are homozygous (Serine/Serine).

European individual ancestry (IA) is associated with subclinical cardiovascular disease phenotypes in African-Americans (AFA) and Hispanics from the Multi-ethnic Study of Atherosclerosis (MESA). *C. L. Wassel[1], J. S. Pankow[1], S. Choudhry[2], C. A. Peralta[2], M. F. Seldin[3], D. K. Arnett[4]* 1) University of Minnesota, Minneapolis, MN; 2) University of California, San Francisco, CA; 3) University of California, Davis, CA; 4) University of Alabama, Birmingham, AL.

Coronary artery calcium (CAC) and common carotid intima media thickness (cIMT) are subclinical measures of atherosclerosis that differ by ethnicity, with AFA in general having larger cIMT but less CAC compared to Caucasians. IA can be an important tool for identifying traits for admixture mapping as well as controlling for population stratification in genetic association studies. Thus, we examined the association of IA with CAC and cIMT among MESA AFA and Hispanics. In 2847 ethnically diverse men and women aged 45-84 from 6 sites in the US, we studied associations of IA with CAC and cIMT using log-binomial and linear regression models, respectively, controlling for confounders. Splines were used to assess the appropriate functional form of IA with CAC and cIMT. CAC and cIMT were measured at baseline with computed tomography and ultrasound, respectively. IA was estimated using STRUCTURE V2.2, which employs Markov Chain Monte Carlo approach. Genotype data was available for 199 ancestry informative markers, and 712 MESA Caucasians, 60 Yoruban Nigerians from HapMap, and 345 Native Americans were used as pseudo-ancestral populations. Among AFA, a standard deviation increase in European IA was associated with an 8% higher CAC prevalence, p=0.02 and a 2% lower cIMT, p=0.008. Average European IA ranged from 15% at the New York site to 25% at the Chicago site. Among Hispanics, the highest tertile of European IA (48.4%) was associated with a 33% higher CAC prevalence compared to the lowest tertile, p=0.02. Average African IA ranged from 4% to 28% across sites, while average Native American IA ranged from 30% to 59% across sites. Higher European IA was associated with CAC and cIMT among AFA and CAC among Hispanics. This suggests that CAC and cIMT may be good candidates for admixture mapping. The variation in IA by site illustrates the importance of controlling for population stratification in genetic association studies.

Bayesian block clustering for multi-trait genome-wide association studies. *M. Gupta[1], Y. H. Hsu[2], S. Demissie[1], L. A. Cupples[1], P. Sebastiani[1], D. P. Kiel[2,3], D. E. Karasik[2,3]* 1) Boston University, Boston, MA; 2) Institute for Aging Research, Hebrew SeniorLife, Boston, MA; 3) Harvard Medical School, Boston, MA.

Many common diseases including diabetes, cardiovascular disease, and osteoporosis are characterized by complex traits, which are determined by the interplay of numerous genetic variants and their interaction with environmental factors. Although genetic and phenotypic data may contain the information to decipher complex diseases, building global models that can associate complex traits with the appropriate genetic profile leads to several formidable statistical and computational challenges. We have developed a novel Bayesian framework for a block clustering model and an efficient Monte Carlo-based computational methodology geared towards identifying sets of candidate genes associated with traits for different diseases, and for prioritizing subsets of candidate genes/SNPs for further evaluation. Our method is suitable for large genetic data sets, and is more appropriate to use than standard clustering techniques when (i) small sets of SNPs are believed to influence a phenotype of interest, or (ii) a single SNP may affect multiple phenotypes that may or may not be influential in all other phenotypic measurements. Application of our method to data from the Framingham Osteoporosis Study shows several interesting SNP-phenotype connections, which illustrate underlying characteristics of bone aging. The data set consisted of 2,005 women and 1,496 men (with a mean age of 62.5 years), members of two generations of Framingham, who were measured for bone mineral density (hip and spine), heel ultrasound, hip and metacarpal geometry. Heritability estimates for all bone health related phenotypes were 30-66%, and some of the phenotypes displayed high genetic correlations.

tRNA splicing endonuclease mutations cause pontocerebellar hypoplasia. *B. S. Budde[1], Y. Namavar[2,3], P. G. Barth[3], B. T. Poll-The[3], P. Nuernberg[1], F. Baas[2]* 1) Cologne Center for Genomics, University of Cologne, Cologne, Germany; 2) Department of Neurogenetics, Academic Medical Center, University of Amsterdam, The Netherlands; 3) Division of Pedriatric Neurology, Emma Childrens Hospital/Academic Medical Center, Amsterdam, The Netherlands.

Pontocerebellar hypoplasias (PCH) represent a group of neurodegenerative autosomal recessive disorders with prenatal onset, atrophy/hypoplasia of the cerebellum, hypoplasia of the ventral pons, microcephaly, variable neocortical atrophy and severe mental and motor impairments. To identify the locus responsible for PCH type 2 we performed a genome-wide scan in two families from the Volendam region in the Netherlands using 10K SNP arrays (Affymetrix). We found linkage to chromosome 17q25 with a maximum LOD score of 5.81. By finemapping using microsatellite markers we narrowed the PCH2 locus to an interval of 3.4 cM between markers D17S1301 and D17S751. We prioritized the genes in this region based on expression pattern and function and sequenced 19 genes. One variant in the tRNA splicing endonuclease homolog 54 gene (c.919G>T) was exclusively homozygous in 47 of 52 analyzed PCH2 cases, all of European descent, but not in the control samples. By genotyping 31 of those PCH2 patients being homozygous for the c.919G>T mutation we found a common SNP haplotype of 285 kb shared by all of them suggesting a founder effect dating at least 11 generations back. Further evidence for a causative role was obtained by extending the TSEN54 mutation screening to our PCH4 patients. In all three patients the c.919G>T variant was found in one of them homozygous while two of them were compound heterozygous carrying a nonsense mutation on the other chromosome. Furthermore we identified mutations in two other of the four different subunits of the tRNA-splicing endonuclease complex in two of the remaining PCH2 cases without the common mutation. Expression analysis of TSEN54 revealed a high level of TSEN54 mRNA in the developing pons, dentate and olive nuclei, which is in line with the PCH2 phenotype. Our findings point to RNA processing as a new basic cellular impairment in neurological disorders.

Genotyping quality of Affymetrix GeneChip Human Mapping 500K Array Set. *G. Zhang[1,2], G. Kennedy[3], J. Schumm[4], R. Chakraborty[1]* 1) Center for Genome Information, Department of Environmental Health, University of Cincinnati, OH; 2) Department of Family Medicine, University of Cincinnati, OH; 3) Genomics Collaborations, Affymetrix Inc, CA; 4) The Bode Technology Group, VA.

The Affymetrix GeneChip Human Mapping 500K Array Set enables high throughput genome-wide SNP analysis and has been widely used in genome-wide association and population genomics studies. It has been shown that even moderate genotyping error rates can have serious affects on haplotype or LD measure inferences and in turn inflate the type I error rate of a study. Therefore, it is important to learn whether the 500K Array Set will provide accurate and robust genotyping calls for DNA samples in practice, which may have large variations in quality and quantity. To answer this question, we analyzed the genotyping quality of the 500K Array Set using the genotype data of 43 forensic samples as well as the data of the 270 HapMap samples. The experimental procedures of the GeneChip Assay generally involve two steps: 1) whole genome sampling analysis (WGSA) by restriction fragmentation and PCR amplification and 2) SNP allele discrimination by probe hybridization. Accordingly, we investigated the impacts of length and Tm of restriction fragments and probe specificity on the genotyping quality of 500K markers, using the observed call rate and confidence score as quality measures. Our results indicate that both length and Tm of restriction fragments could influence the genotyping quality to a certain degree (especially in forensic samples). Also, probe specificity characteristics (i.e. additional homologous hits, known CNV or variation in probe target region) significantly deteriorate genotyping quality. In addition, genotyping quality substantially decreases for markers with low minor allele frequencies. These observations suggest that a subset of Mapping 500K markers with undesirable properties could be pre-filtered from genome-wide data analysis and some marker-specific features should be considered in future SNP array design.

***CFTR* Val470 allele is associated with higher reproductive rates and larger families in the absence of IVS8 5T allele in fertile men.** *G. Kosova[1], C. Ober[1,2,3]* 1) Commitee on Genetics; 2) Dept. of Human Genetics; 3) Dept. of OBGYN, University of Chicago, Chicago, IL.

Congenital bilateral absence of the vas deferens (CBAVD), resulting in male infertility, is considered a primarily genital form of cystic fibrosis, due to the high frequency of cystic fibrosis transmembrane regulator (*CFTR*) mutations in men with CBAVD. The most common mutation leading to CBAVD is the 5T variant at the 3 splice acceptor site of intron 8, which induces alternative splicing of exon 9 and reduced expression of CFTR mRNA. However, linkage disequilibrium between the 5T allele and the Val allele at the Met470Val SNP in CBAVD men, but not in controls, suggests a contribution of Val470 to disease penetrance of CBAVD. On the other hand, the derived Val470 allele resides on an extended haplotype (Pompei et al., EJHG 2006; 14:85-93) and occurs at high frequency only outside of Africa (F_{st} = 0.44 between HapMap CEPH and Yoruba populations), suggesting recent positive selection. Here, we examined fitness effects of the Met470Val polymorphism in fertile couples by considering two measures of fertility, family size and reproductive rate (calculated as 1/mean interbirth interval), in 142 Hutterite men and 157 Hutterite women. The Hutterites, a founder population of European descent, proscribe contraception and desire large families. As a result, mean family size is 7.07 and reproductive rates are among the highest observed in humans. Using an association test that accounts for the relatedness between all pairs of Hutterites, we found that Hutterite men carrying one or two copies of the Val470 have significantly higher reproductive rates (P = 0.00017) and larger family sizes (P = 0.0020), consistent with a selective advantage conferred by this allele. There was no association in women. Furthermore, the IVS8 5T locus allele is absent in the Hutterites and neither the 7T nor 9T alleles was associated with either fertility trait (P 0.10). We suggest, therefore, that the Val470 allele increases male fitness on genetic backgrounds that lack the 5T allele but may act as a modifier to increase the penetrance of the 5T allele in CBAVD. Supported by NIH grant HD21244.

Impact of analyzing multiple contingency tables per SNP on the type I error rate and power in GWAS. *A. G. Matthews[1], J. Ott[1,2]* 1) Ott Lab, Box 192, Rockefeller Univ, New York, NY; 2) Beijing Institute of Genomics, Chinese Academy of Sciences, Beijing, China.

One common approach to the analysis of genome-wide association studies is to consider multiple contingency tables for each SNP. For example, computing the genotype-based test and allele-based test, then reporting the test with the smallest p-value. Alternatively, one may report the test with the smallest p-value over three genetic models: dominant, recessive or heterozygotic susceptibility. We demonstrate via simulation that these two approaches inflate the type I error rates to over 10% in some cases. The power of these methods are also compared indicating that, for most genetic models, once the false positive rate is controlled at 5%, these two methods are as or more powerful than use of the genotype- or allele-based tests alone. Further we apply these methods to a genome-wide association study of Parkinsons disease (Fung et al. 2006) to illustrate the effects of not accounting for the inflation of the type I error rate.

Approaches and Considerations for Applying Random Forests to Genome Wide Data. *B. A. Goldstein[1], A. Hubbard[1], A. Cutler[2], P. P. Ramsey[3], L. F. Barcellos[3], International Multiple Sclerosis Genetics Consortium* 1) Division of Biostatistics, Univ California, Berkeley, Berkeley, CA; 2) Department of Mathematics and Statistics, Utah State University. Logan, Utah; 3) Division of Epidemiology, Univ California, Berkeley, Berkeley, CA.

As computational power improves, the application of more advanced machine learning techniques for the analysis of large genome wide datasets becomes possible. While most traditional statistical methods of analysis can only elucidate potential main effects of genetic variants on disease risk, machine learning approaches are particularly suited to discover interactions and higher order effects. One such approach is the Random Forests (RF) algorithm. Recent years have shown a growth in utilization of RF for SNP discovery in complex disease studies. However, most work has focused on small or simulated datasets which are limited. Using 500K SNP genotypes comprised of 931 multiple sclerosis (MS) cases and controls matched on gender, we outline an approach for optimally tuning RF parameters based on empirical analyses. Our results indicate that the out-of-bag error derived from a small number of trees (~50) can be used to pre-select the optimal number of SNPs to choose at a given node. We also present considerations for addressing issues such as low minor SNP allele frequency and high linkage disequilibrium; both can bias RF analyses. Finally, we show that while RF detects those SNPs with the largest marginal chi-square statistics (top 10), there is considerable lack of overlap between those identified by both RF and a chi-square test among the top 100 (~33%-50%). These results suggest that novel interactions among SNPs may be contributing to MS susceptibility. We also discuss approaches for fully characterizing these potential gene-gene interactions. Acknowledgments: International MS Genetics Consortium (MS case data) and National Institute of Mental Health (control data).

Genetic profiles of various gene-associated subtypes of Myofibrillar myopathies in an International cohort of MFM patients. *H. Lee[1], A. Shatunov[1], M. Olive[2], B. Goudeau[3], N. Sambuughin[4], Z. Odgerel[1], M. Dalakas[5], P. Vicart[3], L. Goldfarb[1], The international MFM Collaborative Group* 1) NINDS, NIH, Bethesda, MD; 2) Ciutat Sanitària i Universitària de Bellvitge, Barcelona, Spain; 3) Université Paris 7, Denis Diderot, Paris, France; 4) Uniformed Services University of the Health Sciences, Bethesda, Maryland; 5) Thomas Jefferson University, Philadelphia, PA.

Myofibrillar myopathies (MFMs)is a group of heterogenic disorders having in common myopathological features of disintegration of myofibrils and accumulation of degradation products in inclusions containing desmin and other myofibrillar and ectopic proteins. A strong feature that brings these disorders into a single disease entity is the fact that the myofibrillar disintegration begins at the Z disc of the sarcomere. Mutations in genes encoding Z disc associated proteins DES, CRYAB, MYOT, ZASP, and FLNC cause MFM. Accumulating data suggest some gene-dependent clinical and myopathological differences between the MFM subtypes. We studied 207 patients from 135 families originating from 22 countries. Patients with MFM were systematically identified, studied and documented at regional referral centers specialized in neuromuscular disorders. Sequence analysis of the above five genes led to the identification of a causative mutation in 91 affected families (67%). DES mutations were detected in 59 (44%), CRYAB mutation in one (0.7%), MYOT in 26 (19%), ZASP in 4 (3%), and FLNC in one (0.7%). Negative screening results in 44 MFM families (33%) suggest the possibility that other genes are involved in MFM. Our results also show the necessity of population specific genetic screening strategy as different sets of genes are involved in different countries: the absolute majority of MFM affected families in Spain show MYOT mutations suggesting existence of a founder effect in the region.

Ethical, Societal, and Policy Implications of Research: Do Geneticists Views Differ from Other Scientists? *J. McCormick, A. Boyce, M. Cho* Stanford Ctr Biomedical Ethics, Palo Alto, CA.

Genetics is an area of scientific inquiry that is viewed to have tremendous impact on society, with the potential to raise complex ethical, legal, and social issues. It has been nearly 20 years since the establishment of the Ethical, Legal, and Social Implications (ELSI) program of the Human Genome Project, in which philosophers, lawyers, social scientists, and others helped to identify and study ethical issues in genetic research. However, we know little about the views of geneticists on the broader ethical, societal, and policy implications of their research. What considerations do they give to these issues, and how do they view the relationships between science and society? Are geneticists more interested in talking about ESP issues than other scientists? To the public? To policymakers? We conducted a mixed methods study, including 16 phone interviews and 5 focus groups with researchers from one institution and survey of 2000 researchers at 7 US institutions. Our sample included researchers from range of positions and departments, including genetics. Geneticists in general share the views of other life scientists. 46% are at least moderately interested in having more opportunities to discuss ethical and societal issues and 16% agree that scientific papers should include a discussion of the ethical and societal implications of the reported data. 25% agree that they have sufficient opportunities to communicate with the public while over 75% agree that it would be better for science if researchers communicated more with the public. The response is mixed on whether involvement of scientists in partisan politics harms the credibility of science. A majority agree that it would be beneficial to science if researchers learned more about the policy-making process and that better science policy would be formed if they interacted more with policymakers. Our data suggest that most researchers recognize the importance of incorporating ELSI considerations in research and participating in discussions about these issues with the public. Furthermore, our findings suggest that there is a role for the ELSI and bioethics community in facilitating these discussions.

Comparison of Family Healthware and Physicians' Family History Documentation Among 1124 Patients. *S. M. O'Neill, E. J. Starzyk, R. A. Kattezham, W. S. Rubinstein* Dept Medical Genetics, Evanston NW Healthcare, Evanston, IL.

Background: Family Healthware (FH) is a web-based tool created by the CDC that collects family history (FHx) of 6 diseases, Coronary Heart Disease (CHD), Stroke (CVA), Diabetes (DM), and Colorectal (CRC), Breast (BC) and Ovarian (OC) cancers and generates family history-based risk assessments and risk-based prevention messages. The Family Healthware Impact Trial (FHITr) is assessing its clinical utility in primary care. Method: In a subset of 1124 patients enrolled at Evanston Northwestern Healthcare, we compared the FHx they entered directly into the FH tool with existing documentation in their medical charts. FH records relationship, disease status, and age of onset for first and second degree relatives. We reviewed charts for the same information. If adequate information was present (at least one affected relative or notation of no family history), it was entered into FH to produce a chart-based risk assessment (CRA). We then assessed the agreement between the direct and CRA risk levels. Results: There was no FHx documentation or insufficient information to perform a CRA in many of the charts: CHD (37.5%), CVA (37.5%), DM (63.5%), CRC (47.7%), BC (51.2%), and OC (40.2%). Of these participants unable to be assessed by chart review, 23% had a moderate or strong risk for one of the diseases when they entered their FHx in FH themselves. For those with chart documentation sufficient to generate a CRA, agreements between risks are: CHD (percent agreement=53%, weighted kappa= 0.399), CVA (60%, 0.338), DM (71%, 0.563), CRC (92%, 0.740), BC (84%, 0.666), and OC (91%, 0.531) ($p<0.04$). FHx recorded directly by participants resulted in higher risk levels ($p<0.05$), with more affected first and second degree relatives and more ages of onset than the FHx documented in their charts. Conclusion: Primary care physicians are not systematically documenting FHx of the 6 common diseases included in the Family Healthware tool. Incorporation of automated family history-based risk assessment tools into clinical practice may increase the opportunity to identify those individuals who would benefit from enhanced surveillance and prevention measures.

3-Methylglutaconic (3MGC) Aciduria in Smith-Lemli-Opitz Syndrome (SLOS). *J.-B. Roullet, L. Merkens, A. Pappu, M. Jacobs, W. Connor, R. Steiner* Oregon Health & Science University, Portland, OR.

SLOS is an autosomal recessive multiple malformation/mental retardation syndrome caused by deficiency in 7-dehydrocholesterol reductase (DHCR7), the last enzyme in cholesterol synthesis. The pathophysiology of SLOS is incompletely understood and there is no proven treatment. In SLOS, cholesterol is reduced while 7DHC accumulates. In addition the urinary excretion of non-sterol isoprenoids (e.g. Coenzyme Q, CoQ) is elevated while that of mevalonic acid (UMVA), the early precursor of cholesterol is unaltered. We hypothesized that due to the distal block in cholesterol synthesis, MVA is diverted toward other pathways including synthesis of 3MGC via the Popjak shunt. We also hypothesized that dietary cholesterol supplementation would decrease U3MGC via decreased MVA synthesis. In 1995, Kelley *et al.* reported that plasma 3MGC is elevated in SLOS, but urine excretion may give a more accurate estimate of diversion. To further explore MVA diversion, we measured 24-hr urinary excretion of 3MGC by GC-MS in 10 SLOS patients on low (LC) or high (HC) cholesterol diet, and in 18 controls (Ctrl). UMVA and UCoQ were also measured (radio-enzymatic assay & HPLC). U3MGC (mol/mol creatinine) was increased in SLOS compared to Ctrl (LC: 173; Ctrl: 9.51.4; $p<0.015$), but was not affected by cholesterol intake (HC: 13.01.7; NS vs. LC). Similarly UCoQ was increased in SLOS (LC: 25.717.1; Ctrl: 3.01.8; $p<0.045$) and not affected by cholesterol intake. UMVA was similar in all groups. There was no correlation between U3MGC and any of the following: plasma cholesterol, 7DHC, 7DHC/Chol ratio, UCoQ or severity score. However, U3MGC was positively correlated with age (r=0.54; $p<0.003$), body weight (r=0.47; $p<0.01$) and UMVA (r=0.65; $p<0.001$). These results demonstrate that SLOS is associated with 3MGC aciduria. The lack of effect of diet on U3MGC and absence of correlation between U3MGC and UCoQ excretion suggests that increased U3MGC in SLOS is not caused by increased shunt activity but by a derangement of mitochondrial energy metabolism, the other known cause for 3MGC aciduria. Such possibility will need to be explored in the future as it may have important treatment implications.

Exact Power Calculations when testing Hardy-Weinberg Equilibrium in Small Samples. *S. Venkatesan, R. Chakraborty, M. Rao* Center for Genome Information, University of Cincinnati, Cincinnati, OH.

The genotype probability distribution for a biallelic marker is generally represented by a 2 X 2 symmetric matrix with identical marginal row and column probabilities. There are many tests available to test Hardy-Weinberg equilibrium (i.e., alleles independently combining to form genotypes). The power of these tests has been examined extensively in literature for large samples. We present a novel approach to calculate power in small samples. The set of all 2 X 2 symmetric distributions with fixed marginals is a compact convex set. This set has precisely two extreme points. The exact distribution of any test statistic under products of extreme points can be determined reasonably well. We demonstrate the calculation of power by cobbling these exact distributions. A comparison of several test statistics in terms of power is made.

ZNF217 is associated with coronary artery disease in multiple samples. *T. Wang[1], B. S. Sutton[1], S. Nelson[1], D. R. Crosslin[1], S. G. Watson[1], D. Seo[3], S. H. Shah[1,2], P. Goldschmidt-Clermont[3], S. G. Gregory[1], W. E. Kraus[1], E. R. Hauser[1]* 1) Center for Human Genetics, Duke University Medical Center, Durham, NC; 2) Division of Cardiovascular Medicine, Duke University Medical Center, Durham, NC; 3) Miller School of Medicine, University of Miami, Miami, FL.

Zinc finger protein 217 (ZNF217) locates to the linkage peak on chromosome 20q13 from our family based linkage study (GENECARD) of early-onset coronary artery disease (CAD). Additionally, a study of gene expression signatures from human aortas identified ZNF217 to be differentially expressed in aortas with and without atherosclerosis. ZNF217 is known to repress the transcription of many genes and is associated with cell proliferation, survival, and invasion. We investigated the role of ZNF217 as a candidate gene for CAD in three independent CAD samples: GENECARD families (n= 1,101 families, 2,934 individuals), CATHGEN case-control samples (cases = 912 and controls = 401), and human donor aorta samples (n = 193). We genotyped 18 single nucleotide polymorphisms (SNPs) based on their linkage disequilibrium (LD), function and tagging potential. Single SNP analysis using logistic regression identified seven SNPs that were significantly associated with CAD in at least one dataset (p-values 0.005 - 0.04). Four of these SNPs are coding (C133C, A342A, I739V, and G899D), including two of the most significantly associated SNPs (A342A and G899D). To further test the potential functional impact of the SNPs, we evaluated allele-specific expression in the aorta samples adjusted for sex, race and age. The results indicated that seven SNPs were significantly associated with the level of ZNF217 expression (p-values 0.004 - 0.04). Two of these SNPs (A342A and I739V) were also significantly associated with CAD in CATHGEN sample. These combined results from independent genome-wide linkage, association, and gene expression studies suggest that ZNF217 is a novel susceptibility gene for CAD. We are currently studying the genes specific contribution to heart disease through the identification of its target genes.

Variants in CAST, the gene encoding Calpastatin, associate with variation in lung disease severity in cystic fibrosis (CF). *V. K. Doshi[1], L. Vanscoy[1], S. M. Blackman[3,1], J. M. Collaco[2,1], L. Bremer[1], G. R. Cutting[1]* 1) Inst of Genetic Medicine, JHMI, Baltimore, MD; 2) Pediatrics Pulmonary Dept, JHMI, Baltimore, MD; 3) Pediatric Endocrinology Dept, JHMI, Baltimore, MD.

Studies of CF twins and CF siblings indicate that genetic modifiers make a substantial contribution to variability in lung disease. Linkage analysis performed using 345 affected sibpairs from 299 families with at least one lung function measurement identified two loci for both cross-sectional and longitudinal lung function measures on chromosome 5 (93 cM and 196 cM). Adjustment of lung function measurements for variation in nutritional status demonstrated that linkage to the locus at 93 cM was influenced by body mass. To determine if variants in a specific gene were responsible for the variation in lung function and BMI, we selected tagged SNPs from 22 candidate genes within the linkage locus on chromosome 5 (~80-100cM). We then analyzed for association between individual SNPs and patterns of SNPs (i.e. haplotypes) in 562 patient-parent-parent trios using family based association testing (FBAT). Two SNPs (rs27654 and rs27980) demonstrated association for cross-sectional and longitudinal measures of lung function (p values ranging 0.04-0.0004). These SNPs were located in the 3 region of CAST, the gene that encodes calpastatin. The two SNPs demonstrate linkage disequilibrium with each other and haplotypes constructed of these SNPs fall into two major classes accounting for 66% and 33% of genetic diversity in CAST. The common haplotype is associated with lower lung function (AvgFEV1CF%, Z=-3.4, p=0.0007) while the minor haplotype is equally associated with improved lung function (AvgFEV1CF%, Z= 3.4, p=0.0007). Genotype analysis of these CAST variants show that mean lung function measures of homozygotes for the minor alleles of either SNP differ from those homozygous for the major alleles (e.g. rs27980: AvgFEV1CF% 0.47 vs. 0.22, p=0.015). Intriguingly, genetic variation in CAST has been associated in several mammalian species with differences in skeletal muscle mass and repair. These analyses support CAST as the first genetic modifier of CF identified using positional methods.

Candidate genes in antisocial personality disorder (ASPD), an association study with variants in genes involved in both dopaminergic and serotoninergic system in prison population in Antioquia. Colombia. *M. Cuartas[1,2], C. Palacio Acosta[2], Y. Garcia Valencia[1,2], C. Mejia[2], G. Montoya[2], C. Lopez Jaramillo[2], J. Arango[2], Y. Caicedo Petrovich[1], P. Montoya[2], G. Bedoya Berrio[1],* *GRANT 1115-04-16373 COLCIENCIAS* 1) Molecular Genetics Group (GENMOL), Biology Institute, Antioquia Univ, Medellin, Colombia; 2) Dept Psychiatry, Medicine School. Antioquia Univ. Medellin, Colombia.

Background: many studies haves implicated the dopamine and serotonin neurotransmission in psychiatric disease. Several genetic association studies have been published, but so far, no DNA variants have been unequivocally demonstrated as contributing to ASPD susceptibility. Dopamine and serotonin related gene loci have been implicated; however, each of these may influence the liability of ASPD to a small degree. Notably, all are involved in signal transduction at the neuronal synapse. In this propose, we investigate as candidate genes for ASPD, DNA polymorphisms at dopamine and serotonin receptors, and genes known to be involved in both systems. Methods: a case-control study,530 individuals, male population from prison population in Antioquia, Colombia. The clinical evaluation used: semi structure clinical interview, DSM IV criteria, DIGS and PCL (Check list). We genotyped eight snps and five VNTRs strongly associated in previous researches. Results: The present study strongly supports disequilibrium linkage in HTTLPR, TPH1 and HTR2A; moreover, the COMT was significantly associated with ASPD. The markers DRD4, DRD2 and DAT1 were related with spectrum disorder such as drugs, addiction and alcoholism. Conclusions: despite the limited sample size, these results promote the notion that genotype and psychosocial factors interact to precipitate male adult criminal behavior; and show implications for the design of future association studies of personality disorders, including the likely sample sizes that will be required to achieve sufficient power and the potential role of moderating variables like as cognitive endophenotypes.

The DNA replication FoSTeS mechanism can cause human genomic, genic, and exon shuffling rearrangements.

F. Zhang[1], M. Khajavi[1], J. R. Lupski[1,2,3] 1) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX 77030; 2) Department of Pediatrics, Baylor College of Medicine, Houston, TX 77030; 3) Texas Childrens Hospital, Houston, TX 77030.

We recently proposed a DNA replication based mechanism of Fork Stalling and Template Switching (FoSTeS) to explain the complex rearrangements associated with Pelizaeus-Merzbacher disease (PMD), an X-linked dysmyelinating central nervous system disorder. The hallmarks of FoSTeS are complex rearrangements that result from template driven juxtaposition of sequences from different genomic locations with DNA sequence microhomology at the join point(s). Here we provide further evidence for human genomic rearrangements generated by FoSTeS and the involvement of autosomal loci in which such rearrangements convey genomic disorders including Potocki-Lupski syndrome (PTLS) and Charcot-Marie-Tooth disease type 1A (CMT1A). We now show by oligonucleotide array CGH and breakpoint sequencing that 8/14 nonrecurrent PTLS associated duplications are complex and occur by FoSTeS. Furthermore, we show that >99% of CMT1A duplications/HNPP deletions occur by NAHR, but the majority of nonrecurrent rearrangements (6/7) appear to occur by FoSTeS. Our data suggest FoSTeS may be a major mechanism for generating structural variation of the human genome. Furthermore, we show that this mechanism can also apply to both genic and single exon rearrangements indicating a link to gene evolution and potentially the mechanism for the long postulated exon shuffling.

Phenotype /Genotype Correlation and Prevalence determination of Spondylothoracic Dysostosis/ Jarcho-Levin Syndrome. *A. S. Cornier[1], S. Carlo[1], N. Ramirez[2], N. Arciniegas[3], J. Flynn[2]* 1) Dept. of Molecular Medicine, La Concepcion Hospital, San Germán, PR; 2) Dept. of Orthopeadics, La Concepcion Hospital, San Germnán, PR, PR; 3) Dept.of Pediatrics, La Concepcion Hospital, San Germán, PR.

Spondylothoracic Dysostosis (STD) also know as Jarcho-Levin syndrome (JLS), is an autosomal recessive disorder characterized by abnormal vertebral segmentation and formation defects affecting the spine with complete bilateral fusion of the ribs at the costovertebral junction. The shortened spine and trunk may severely affect respiratory function in early childhood. Although STD is prevalent in the Puerto Rican population it is a pan-ethnic disorder. Recently mutations in the MESP2 gene were identified as responsible for the STD phenotype. Molecular characterization of the disease has been achieved in over 35 patients with the disorder including identification of non-sense, missense and deletions within the MESP2 gene. Genotype/phenotype characterization has provided important correlations regarding the severity of the disease for specific mutations. These findings extend the range of abnormal vertebral and rib malformation phenotypes, clinical complications in neonatal period, ventilatory parameters, management, mortality and morbidity for specific mutations in the MESP2 gene. We have performed MESP2 mutation screening in 450 newborn samples and determined a prevalence of the disease of 1 in 6,800 live births. With such a high prevalence we are studying possible beneficial effects for heterozygotes of MESP2 gene mutations. This new molecular, clinical and epidemiological information may prove to be useful for medical management and genetic counseling in Spondylothoracic Dysostosis.

Human Cognition is Related to STX1A Gene Expression. *M. Gao[1,2,3], U. Bellugi[4], L. Dai[7], D. Mills[5], E. Sobel[3], K. Lange[3,6], J. Korenberg[7]* 1) Dept Med Gen, Cedars-Sinai, Los Angeles, CA; 2) Dept. of Pediatrics, David Geffen School of Medicine at UCLA, Los Angeles, CA; 3) Dept. of Human Genetics, David Geffen School of Medicine at UCLA, Los Angeles, CA; 4) Laboratory of Cognitive Neuroscience, The Salk Institute for Biological Studies, La Jolla, CA; 5) Dept. of Psychology, Emory University, Atlanta, GA; 6) Dept. of Biomathematics, David Geffen School of Medicine at UCLA, Los Angeles, CA; 7) The Brain Institute at the University of Utah, Salt Lake City, UT.

Although genetics is the most significant known determinant of human intelligence, specific gene contributions are largely unknown. To accelerate gene discovery, we have studied the relation between gene expression and IQ in a cohort of 67 patients with Williams syndrome, a mental retardation syndrome caused by a 1.5Mb deletion on chromosome 7q11.23. We find that variation in the brain gene STX1A correlates significantly with intelligence $r = 0.40$ ($p = 0.0005$) in WS patients, accounting for 14.9% of variation in the first principal component of the standard Wechsler Adult Intelligence Scale-Revised intelligence test. These results suggest that syntaxin 1A, a neuronal regulator of presynaptic vesicle release, is a component of the cellular pathway determining human intelligence.

Involvement of pathogens in recurrent hydatidiform moles caused by *NLRP7* mutations. *E. Bukhari[1,2], C. Deveault[1,2], J. Qian[1,2,5], A. Mehio[3], L. Gilbert[2], M. Seoud[4], x. Xie[5], R. Slim[1,2]* 1) Human Genetics Department, McGill University Health Center, Montreal, Quebec, Canada; 2) Dept of Obstetrics and Gynecology,McGill University Health Center, Montreal, Quebec, Canada; 3) Dept of Pathology, McGill University Health Center, Montreal, Quebec, Canada; 4) Dept of Obstetrics and Gynecology American University of Beirut, Beirut, Lebanon; 5) Womens Reproductive Health Laboratory, Womens Hospital, Zhejiang University School of Medicine, Hangzhou, China.

Hydatidiform mole (HM) is an abnormal human pregnancy characterized by the absence of, or abnormal, embryonic development and hydropic degeneration of the chorionic villi. Recently, *NLRP7* has been found responsible for recurrent hydatidiform moles (RHM) by the identification of 11 mutations in this gene (Murdoch et al., 2006; Kou et al, 2008). In this study, we investigated the presence of microorganisms in several molar tissues from several patients with NLRP7 mutations by microscopy screening of sectioned tissues stained with Gram and Grocotts methenamine silver and by PCR amplification with universal bacterial and fungal primers followed by cloning and DNA sequencing. Microscopy screening revealed Gram-positive (G+) cocci, Gram-negative (G-) bacilli, yeast cells and filamentous fungi, in most tissues from the patients, while only 10% of control tissues from elective first trimester abortions had a few G+ bacilli and G+ cocci. PCR amplification with universal bacterial and fungal primers followed by cloning and DNA sequencing confirmed the presence of several microorganisms in the patients and the pathogenic nature of some of them. Our data demonstrate the involvement of pathogens in RHMs caused by NLRP7 mutations and will have great impact on our current understanding of the pathology of moles and reproductive wastage.

New testing strategy for first trimester prenatal diagnosis by CVS. *V. Cirigliano[1,2], G. Voglino[3], E. Ordoñez[1,2], A. Marongiu[3], L. Rueda[1,2], C. Mediano[4], C. Fuster[2]* 1) General Lab, Barcelona; 2) Universitat Autonoma de Barcelona; 3) Cytogenetics Lab Promea ,Turin; 4) Hospital Vall dHebron, Barcelona.

Prenatal diagnosis in CVSs usually implies sequential karyotyping cytotrophoblastic cells (STC) and long term cultures (LTC). Main drawbacks of this approach are possible discordance between results obtained on different cell populations, confined placental mosaicism (CPM) and maternal contamination. The great majority of chromosome abnormalities can readily be detected by QF-PCR analysis of microsatellites. However, when applied on CVSs, STRs may also provide valuable information about the presence of different cell lines in mosaic or contaminated samples and the origin of extra chromosomes in trisomic cases. We analysed 3696 consecutive CVSs by QF-PCR and karyotyping; following an initial evaluation on 500 samples, QF-PCR fully replaced the STC. QF-PCR was performed on a single villus within 24 hours from sampling, abnormal results were confirmed on a second independent frond. In alternative, DNA extraction was performed on the cellular pool obtained after digestion prior to cell culture. A total of 201 cytogenetic abnormalities were observed, 98% were detected by QF-PCR with 100% PPV and 98.7% NPV. Five CPM cases could be detected by QF-PCR because of trisomic diallelic patterns observed for all informative markers in single tissue fronds. Six true mosaic fetuses were detected by QF-PCR with STR patterns clearly confirming the meiotic origin of the extra chromosome. Maternal cell overgrowth in 2 LTC could be revealed by the discrepancy with the QF-PCR result of normal male. LTC failed in 3% of cases because of poor sample quality or amount, QF-PCR analysis was always possible and in several cases a second invasive procedure could be avoided. The QF-PCR test coupled with LTC is a robust diagnostic approach. The molecular assay allowed discriminating true fetal abnormalities from CPM in several cases thus avoiding further investigations by amniotic fluid sampling. This testing strategy proved to be efficient and reliable also if applied on samples of limited size and quality and its routine application resulted in a significant reduction of costs, hands on and reporting time.

Cystinuria in Australian Cattle Dogs: A Model of Non-Type I Cystinuria. *P. Henthorn[1], J. Liu[1], A. Shearin[1], J. Westropp[2], A. Huff[1], U. Giger[1]* 1) Section Medical Genetics, Univ PA Sch Veterinary Med, Philadelphia, PA; 2) Dept. of Medicine and Epidemiology, College of Veterinary Med, Univ CA-Davis, Davis CA.

Cystinuria is an inherited transport defect in the proximal renal tubules associated with reduced reabsorption of cystine and other dibasic amino acids and has been documented in > 60 dog breeds. Because cystine at high concentrations precipitates in acidic urine, cystinuria may lead to formation of urinary cystine crystals and calculi that could lead to urinary obstruction. Mutations in the SLC3A1 and SLC7A9 genes, which encode a dibasic dimeric amino acid transporter, are responsible for cystinuria in humans, where the disease is classified clinically as Type I or non-Type I cystinuria based on cystine and dibasic aminoaciduria in obligate heterozygotes. Aminoaciduria is present in obligate carriers of non-type I cystinuria, as is urolithiasis in a small proportion of individuals. Therefore, in humans, Type I cystinuria is inherited as an autosomal recessive trait, while the inheritance pattern of non-Type I cystinuria is best described as autosomal dominant, with incomplete penetrance. In our ongoing study of dogs from different breeds ascertained by formation of cystine calculi or by unusually high urinary cystine levels, we previously characterized cystinuria in Newfoundland and Labrador retriever dogs at the clinical and molecular levels, identifying Type I cystinuria with early onset of calculi formation due to homozygosity of different mutations in the SLC3A1 gene in each breed. Here we report the results of studies of cystinuric male Australian cattle dogs. We sequenced the SLC3A1 gene and found a six bp (two amino acid) deletion in an exon of the SLC3A1 gene in three dogs. Two dogs with stone formation before age one year were homozygous for the deletion, while the dog with later onset of stone formation was heterozygous. There are no amino acid length differences in this exon among diverse species, from zebrafish to man, suggesting that this deletion may have deleterious effects. Therefore, this six bp deletion appears to be analogous to an allele for non-Type I cystinuria in humans, where stone formation can occur in obligate carriers.

Ambroxol as a potential enzyme-enhancement therapy for Gaucher disease. *G. H. B. Maegawa[1,2], M. B. Tropak[1], J. Buttner[1], G. Kornhaber[3], B. Rigat[2], J. T. R. Clarke[1,2], D. J. Mahuran[2]* 1) Div Clin Metab Genet - Peds, Hospital for Sick Children, Toronto, Ontario, Canada; 2) Research Institute, Hospital for Sick Children, Toronto, Ontario, Canada; 3) Exsar Inc., Monmouth Junction, NJ, USA.

Gaucher disease, currently treated by enzyme replacement therapy (ERT), is caused by a deficiency of lysosomal -glucosidase (GCase). The disadvantages of ERT include its high cost, its ineffectiveness in treating the CNS or other organ compartments and its failure to prevent the unfolded protein response in cells. Small molecule-based enzyme enhancement therapy (EET) is a promising approach that can potentially be used alone or in combination with ERT to address these deficiencies. Clinical trials of isofagomine, an inhibitor of GCase (IC50~0.04 M), as an EET-agent are being initiated. In order to accelerate the process of obtaining IND-status for new EET-agents we have screened the NINDS library of FDA-approved drugs for compounds that inhibit and/or stabilize the target enzyme towards heat denaturation. Using GCase as the target we identified ambroxol, an expectorant, as a candidate EET-agent. Despite ambroxol being only a weak inhibitor of GCase, IC50 ~ 27 M, at higher concentrations it compared favorably with isofagomine in its ability to rescue mutant N370S GCase in patients fibroblast and lymphoblast cell lines. However, it was not as effective at rescuing F213I mutant GCase. Cells with wild-type GCase also show a mild enhancement in activity. Hydrogen-deuterium exchange mass spectrometry (H/D-Ex) was used to compare the regions in GCase that were stabilized by these compounds. Isofagomine has been shown through co-crystallization to stabilize a loop structure at GCase311-319. Both compounds were equally effective in stabilizing two additional loop regions at the mouth of the active site, GCase243-249 and 386-400, suggesting its importance as a target for EET-agents.

Improved survival among people with mosaic trisomy 21 compared to standard trisomy 21 and Robertsonian translocations. *C. Svaerke[1], S. A. Rasmussen[2], J. M. Friedman[3], D. Schendel[2], H. Hasle[4], A. Correa[2], P. Thorsen[1]* 1) University of Aarhus, Aarhus, Denmark; 2) Centers for Disease Control and Prevention, Atlanta, GA; 3) University of British Columbia, Vancouver, BC, Canada; 4) Aarhus University Hospital Skejby, Aarhus, Denmark.

Health outcomes among persons with mosaic trisomy 21 have not been well documented. The purpose of this study is to compare mortality rates among people with Down syndrome by karyotype using the population-based Danish registries. People with Down syndrome diagnosed by chromosomal analysis from 1961 through 2007 were identified through the Danish Cytogenetic Central Register. The study cohort was born between 1902 and 2007 and was observed from April 1968 or birth (if born later) until death, emigration, or the end of follow-up (May 2008). Dates of death or emigration were retrieved from the Danish Central Person Register. Mortality rate ratio [MRR] was estimated for mosaic trisomy 21 and Down syndrome associated with Robertsonian translocations using Cox regression, with standard trisomy 21 as the reference group. Year of birth and sex were treated as potential confounders. Among the 3,551 people with Down syndrome identified, 3,287 had standard trisomy 21 (921 deaths), 151 had Robertsonian translocations (38 deaths), and 113 had mosaic trisomy 21 (29 deaths). The MRRs for people with mosaic trisomy 21 were 0.55 (95% confidence interval [CI]: 0.39-0.79) for the full cohort and 0.43 (95% CI: 0.21-0.92) for the cohort born in 1968 or later (a group followed since birth). In contrast, for people with Robertsonian translocations, the MRRs were 1.03 (95% CI: 0.75-1.42) for the full cohort and 0.88 (95% CI 0.56-1.37) for the cohort born in 1968 or later. In conclusion, survival among persons with mosaic trisomy 21 is significant greater than that for persons with standard trisomy 21 or Robertsonian translocations. If this finding is replicated in future studies, this information could be useful to clinicians counselling affected families.

Chromosome 17q21.31 microdeletion in a patient with autism. *C. Betancur[1], D. Moreno De Luca[1], A. Gennetier[1], F. Devillard[2], V. Ginchat[2], B. Assouline[3], C. Gillberg[4], M. Leboyer[5]* 1) INSERM U513, Paris, France; 2) Grenoble University Hospital, Grenoble, France; 3) Centre Alpin de Diagnostic Précoce de lAutisme, Saint Egrève, France; 4) Dept. Child and Adolescent Psychiatry, Goteborg University, Goteborg, Sweden; 5) Dept. Psychiatry, Albert Chenevier Hospital, Créteil, France.

Recent findings suggest that microdeletions/microduplications involved in mental retardation also play an important role in autism. A new 17q21.31 microdeletion syndrome, caused by non-homologous recombination between low copy repeats, was recently described in 11 individuals. The subjects had overlapping deletions of 500-650 kb, developmental delay, hypotonia and characteristic facial features. The purpose of this study was to ascertain the involvement of 17q21.31 deletions in autism. A total of 410 patients with autism spectrum disorders recruited by the PARIS study were screened for deletions and duplications associated with mental retardation syndromes using multiplex ligation-dependent probe amplification (MLPA). We identified one patient with a deletion at 17q21.31. Characterization of the breakpoints with qPCR showed that the deletion was about 900 kb and comprised nine genes, including MAPT and CRHR1. The deletion was not present in the mother; the father was deceased and no DNA was available. Analysis of polymorphisms in the MAPT gene showed that the deletion arose from the H2 haplotype inherited from the father. The H2 haplotype comprises a 900 kb inversion polymorphism which makes it susceptible to rearrangements, and is the origin of all of the deletions described to date. The girl had delayed motor and speech development, as well as mild dysmorphic features similar to the ones described previously, including long face, blepharophimosis, bulbous nasal tip, broad chin and long fingers. At 11 years of age, she fulfilled diagnostic criteria for autistic disorder and had a global IQ of 40. In conclusion, this is the first report of a 17q21.31 microdeletion associated with autism. Although the prevalence appears to be low, this observation adds to the increasing list of genomic disorders involved in the etiology of autism.

Prenatal diagnosis of marker chromosome 4: A report of two cases with outcomes. *D. Myles Reid[1], K. Chong[1], E. Kolomietz[2], R. Hopkin[3], A. Bedard[3], J. Kogan[3]* 1) Prenatal Diagnosis and Medical Genetics, Mount Sinai Hosp, Toronto, ON, Canada; 2) Pathology and Laboratory Medicine, Mount Sinai Hosp, Toronto, ON, Canada; 3) Division of Human Genetics, Cincinnati Children's Hosp Medical Center, Cincinnati, OH.

Marker chromosomes are small pieces of chromosomal material that vary in size and are self-replicating. They commonly contain heterochromatic regions but may contain euchromatin with normal or rearranged structure. We report 2 cases of marker chromosome 4 diagnosed prenatally. Case 1 presented with a positive first trimester screen and amniocentesis to rule out chromosomal abnormalities. Concurrently, chromosome analysis completed on the father due to a family history of MR revealed a mosaic karyotype, 47,XY,+mar[8].ish der(4)/46,XY[32]. Fetal chromosome analysis also revealed a mosaic female karyotype, 47,XX,+mar[11]/46,XX[5]. Microarray analysis on the father and fetus showed an identical marker derived from the pericentromeric region of the short and long arms of chromosome 4. Detailed fetal ultrasound was normal and the remainder of the pregnancy was uncomplicated. Case 2 presented for amniocentesis for advanced maternal age and the results revealed a mosaic karyotype, 47,XX,+mar[15]/46,XX[7]. Microarray analysis performed to better define the marker identified the marker as derived from chromosome 4. The marker was shown to be de novo as parental studies were normal. Detailed fetal ultrasound showed choroid plexus cysts, IUGR and oligohydramnios. Fetal echo and fetal MRI were normal. The markers in Case 1 and Case 2 show significant overlap and are both derived from the pericentromeric region of chromosome 4. Both cases resulted in the delivery of healthy newborns. Postnatal follow up has shown no medical or neurological problems for either infant. These are the first reported cases of prenatally detected marker chromosome 4 with normal postnatal outcomes to date. Interestingly, these cases highlight that various parts of the genome even in large imbalance may not significantly impact normal development in humans.

Genome-wide association confirms variants in *SLC2A9* are associated with serum uric acid in Mexican Americans. *VS. Voruganti[1], JW. Kent Jr.[1], SA. Cole[1], M. Carless[1], JE. Curran[1], MP. Johnson[1], HH. Goring[1], L. Almasy[1], TD. Dyer[1], NH. Arar[2], R. Bauer[2], JW. MacCluer[1], HE. Abboud[2], AG. Comuzzie[1], EK. Moses[1], J. Blangero[1]* 1) Southwest Foundation for Biomedical Research, San Antonio, TX; 2) University of Texas Health Science Center at San Antonio, San Antonio, TX.

Increased serum uric acid (SUA) is a risk factor for gout, renal and cardiovascular disease and is known to aggregate in families. Variation in SUA is controlled by both genetic and environmental factors. The purpose of this study was to identify genetic factors that affect the variation in SUA levels in Mexican Americans of the San Antonio Family Heart Study. We used a linear regression-based association test under an additive model of allelic effect, while accounting for the non-independence of family members via a kinship variance component. All analyses were performed in SOLAR. In 848 subjects with SUA measures, mean SE age was 47.87 14.8y. SUA levels were higher in men (6.11.7 mg/dl; n= 313) than in women (4.941.6 mg/dl; n=535). SUA was significantly heritable (h^2 = 0.39 0.07, p=2.3 x 10^{-8}). A genome-wide association (GWA) analysis was performed in 605 subjects using the Illumina Human Hap 550K single nucleotide polymorphism (SNP) chip. SNP rs6832439 within the solute carrier family 2, member 9 (*SLC2A9*) gene was associated with SUA levels at genome-wide significance (p = 7.2 x 10^{-8}). The minor allele of this SNP had frequency of 35.5% and was associated with decreasing SUA levels. The *SLC2A9* gene encodes a urate transporter that has significant influence on SUA levels. Three additional SNPs rs737267 rs6449213 and rs13131257 showed suggestive association (p < $6.8x10^{-7}$) with SUA levels. Suggestive associations were also found in *DRD5* (~ 200kb from *SLC2A9*) and in *ACTL8* on chromosome 1. Our finding replicates a reported association of SUA with this gene in European populations in Germany, Austria, Scotland, Croatia, Sardinia and the United Kingdom. SNPs rs6832439 and rs737267 are in intron 7, the location of the previously reported associations. SNPs rs6449213 and rs13131257 are in introns 4 and 5, respectively. In summary, we have replicated the association of *SLC2A9* with SUA in a Mexican American cohort.

***PPP1R3B* as a Candidate Gene for Congenital Diaphragmatic Hernia.** *N. D. LopezJimenez[1], A. Moshrefi[1], G. Shaw[2], A. M. Slavotinek[1]* 1) Pediatrics, UCSF, San Francisco, CA; 2) C.H.O.R.I., Oakland, CA.

Congenital diaphragmatic hernia (CDH) is a common, life threatening birth defect. A 5-6 Mb chromosome region required for diaphragm formation has been mapped to 8p23.1, between D8S1706 at 6.83 Mb and RP11-252C15 at 12.59 Mb (Shimokawa et al., Am J Med Genet 2005;136:49; Slavotinek et al., J Med Genet 42:730). This region contains the *PPP1R3B* gene which we selected for further study because *in vitro* studies in L6 rat skeletal muscle cells depleted for PP-1G, the encoded protein, show an inability to differentiate (Ragolia et al., J Biol Chem 2000;275:26102). Sequencing of *PPP1R3B* in 96 CDH patients revealed c.481delG in an Hispanic male with left CDH, pectus excavatum and cryptorchidism. This substitution predicts a premature stop at amino acid 162 in the CBM_21 domain of the protein, suggesting haploinsufficiency. The variant was not found in more than 100 Hispanic control chromosomes. However, it was present in the probands normal mother. In-situ hybridization studies with murine sections did not confirm *Ppp1r3b* expression in the diaphragm and we also could not detect expression in proliferating or differentiating C2C12 myoblasts. We therefore did not establish a relationship between this gene and CDH. However, *PPP1R3B* is known to enhance the rate at which PP-1G activates glycogen synthase, and homozygous null mice for *Ppp1r3b* have impaired glycogen accumulation in muscle. Linkage to chromosome 8p23 has been described in both MODY and type II diabetes and PPP1R3B has also been sequenced in 13 predominantly Caucasian families with type 2 diabetes or MODY linked to 8p, but no pathogenic variants were observed (Dunn et al., Ann Genet 2005;70:587). It is therefore interesting to speculate that the c.481delG mutation could cause metabolic effects relating to impaired glycogen synthase. The maternal grandmother had type 2 diabetes in her sixth decade, but DNA was not available. The proband also had significant feeding difficulties with failure to thrive at 34 months, requiring a G-tube. However, further studies are needed to determine if this *PPP1R3B* sequence variant can induce significant metabolic effects.

Amplification Distortion Test: A Method to Fine Map Selection in Tumors. *N. Dewal[1], M. Freedman[2], T. LaFramboise[3], I. Pe'er[1]* 1) Columbia University, New York, NY; 2) Dana Farber Cancer Institute, Harvard Medical School, Boston, MA; 3) Case Western Reserve University, Cleveland, OH.

Selection of amplified genomic segments in particular somatic cellular lineages drives tumor development. However, pinpointing genes under such selection has been difficult due to these regions large sizes. We propose a new method, called the Amplification Distortion Test (ADT), that identifies specific nucleotide alleles that confer better survival for tumor cells when somatically amplified. ADT draws upon the Transmission Disequilibrium Test from statistical genetics and is extended to evaluate and localize distortion on haplotypes in addition to single markers. It is optimized for performance and includes computational techniques to address the intricate challenges of discerning true biological signals from technology-induced artifacts in data. ADT is used to analyze our pioneering dataset, containing 700 tumor samples from lung cancer patients that are typed for copy number variation and 240K SNPs genome-wide. We detect candidate single-SNP and haplotype distortion signals on multiple chromosomes, thus revealing prime target regions for fine mapping. We propose that this novel mode of genome scanning via ADT constitutes a new paradigm for mapping oncogenes. Analogous datasets currently being accumulated by other organizations, such as the Cancer Genome Atlas, provide compelling avenues for further investigation and application of this new methodology.

A functional missense variant in glutamate transporter EAAT1 in Tourette syndrome. *A. Adamczyk[1], C. D. Gause[2], R. Sattler[3], S. Vidensky[3], J. D. Rothstein[2,3], H. Singer[2,3], T. Wang[1,2]* 1) Inst Genetic Medicine, Johns Hopkins Univ, Baltimore, MD; 2) Dept. of Pediatrics, Johns Hopkins Univ, Baltimore, MD; 3) Dept. of Neurology, Johns Hopkins Univ, Baltimore, MD.

Tourette syndrome (TS) is a neuropsychiatric disorder characterized by the presence of involuntary motor and phonic tics. TS has an estimated prevalence of about 1% in school age children. A genetic etiology is supported by large family and twin studies, although the specific genetic abnormality remains undetermined. Pathophysiologically, evidence suggests an abnormality localized within cortico-striatal-thalamo-cortical pathways and possibly an abnormality of dopamine neurotransmission. Glutamate is the major excitatory neurotransmitter in the CNS and has wide interactions with dopaminergic pathways. Glutamate levels are tightly regulated in the synaptic cleft by Na+-dependent glutamate transporters. The goal of this study was to explore the genetic contribution of glutamate signaling in the pathogenesis of TS. DNA sequence variants in EAAT1, which encodes the main glutamate transporter in astrocytes, was evaluated in a well-characterized cohort of TS patients (n=250; TS-only, 106; TS plus ADHD, 148) and controls (n=217) (Yoon et al, 2007). All exon-containing regions of EAAT1 were screened using CE-SSCP and sequenced. A missense variant, E219D, was identified in 11 heterozygous individuals with TS and 4 controls. Allele frequency for E219D was 2.4 and 3.1 times higher in the total TS (0.022) and TS-only (0.028) cohorts, respectively, as compared to controls (0.009). E219 is a residue that is highly conserved from human to x-tropicalis. In order to pursue a functional association with the presence of the E219D variant, a 3H-glutamate-uptake assay was performed in transfected HEK293 cells. Results showed that E219D conveys a significant increase in the EAAT1-mediated glutamate uptake as compared to the wt control. These results raise the possibility that the E219D variant and its alteration of glutamate uptake may have a pathogenic role in a small number of individuals with Tourette syndrome. The role of glutamate signaling in Tourette syndrome warrants further investigation.

Extent of Copy Number Variation in an isolated population of European descent. *H. Xi[1], G. Sun[1], S. R. Indugula[1], G. Zhang[1], P. Rudan[2], R. Chakraborty[1], R. Deka[1]* 1) Dept Environmental Health, Univ Cincinnati, Cincinnati, OH; 2) Institute for Anthropological Research, Zagreb, Croatia.

Copy Number Variation (CNV) has emerged as an important class of genetic variation influencing genetic traits. However, the extent of CNV in worldwide populations has yet to be fully ascertained. We have constructed a CNV map based on data from 235 individuals from a relatively isolated island population from the eastern Adriatic coast of Croatia. Primarily of Slavic origin, the population migrated to the Adriatic islands in the 15th century. DNA samples were genotyped using the Affymetrix SNP Array 5.0 that includes ~500K SNPs and 420K non-polymorphic probes for detection of CNVs. We used two packages for data normalization and copy number segment identification - Copy Number Variation Module (CNAM) of Golden HelixTree and Bengtssons R-package aroma.affymetrix. Our results show ~10% of the genome is covered by CNVs; average length of CNV regions is 45.1Kb with a median length of 1.4Kb. Additional data on >600 samples are currently being analyzed for a finer resolution of CNVs in this population. Supported by grant R01DK069845.

Canine Models of Ichthyosis. *M. L. Casal[1], E. A. Mauldin[2]* 1) Sect Medical Genetics, Univ Pennsylvania, Philadelphia, PA; 2) Dept Pathobiology, Univ Pennsylvania, Philadelphia PA.

Ichthyoses are characterized by faulty formation of the outer layer of the epidermis, the stratum corneum, with resultant scaling and include a heterogeneous group of hereditary and congenital diseases in humans. In dogs, relatively few ichthyosiform disorders have been documented and most are reported as single cases. Here we present two forms of non-epidermolytic ichthyosis in the golden retriever and the American bulldog. The golden retriever disease is characterized by mild to moderate, generalized, nonpruritic scaling (large, loosely-adherent, soft, white to gray scales) with clinical lesions becoming apparent between 8 weeks and 2 years of age. American bulldogs typically have a more severe phenotype with lesions evident at birth or shortly thereafter. The scaling is generalized with large, light brown, plate-like, adherent scale on the ventral thorax and abdomen. The puppies may develop chronic pruritus that coincides with the onset of secondary malassezial infections. Histopathologic findings are similar in both disorders: laminar orthokeratotic hyperkeratosis with an absence of epidermal hyperplasia and dermal inflammation. Ultrastructural analysis using a ruthenium tetroxide fixation method revealed retained and convoluted membranes with crystalline structures in the stratum corneum in both breeds of dogs. Scattered keratinocytes in the granular cell layer had prominent, clear, membrane bound, cytoplasmic vacuoles. Pedigree analyses in both breeds are highly suggestive of an autosomal recessive trait. Molecular characterization is underway for both forms. In humans, many forms of ichthyosis can be debilitating and require lifelong treatment which is only symptomatic. Here we offer a large animal model for treatment trials that will hopefully lead to curative therapies for humans affected with ichthyosis.

A family with Rapp-Hodgkin-like syndrome with intellectual disability and a microdeletion at 13q31.3 that includes the *GPC5* gene. *R. Aul[1], P. Eydoux[2], D. Chai[3], M. I. Van Allen[1]* 1) Medical Genetics; 2) Pathology & Laboratory Medicine; 3) Canadian Molecular Cytogenetics Network, University of British Columbia, Vancouver, BC.

Rapp-Hodgkin syndrome (RHS) is an autosomal dominant condition with anhydrotic ectodermal dysplasia, cleft lip and palate and a specific facies. There is a great deal of clinical overlap with other ectodermal dysplasias including Hay-Wells syndrome and EEC syndrome. These conditions are allelic to mutations in *TP63* gene, the only gene shown thus far to be associated with RHS. We are reporting a family of three siblings and their mother, who have autosomal dominant inheritance of microcephaly, developmental delay, and ectodermal dysplasia features consistent with RHS. The proband is a 13 year old boy with microcephaly and moderate developmental delay. The features of ectodermal dysplasia in him include hypodontia with conical-shaped teeth, enamel hypoplasia, anhydrosis, fine hair, weak nails and abnormal clavicles. In addition, he was born with a cleft lip/palate suggesting a diagnosis of RHS. His mother had a history of learning disabilities as well as anhydrosis, hypodontia, conical teeth, enamel hypoplasia, sparse hair and weak nails. The proband has two older siblings also presenting with microcephaly, learning disabilities and similar ectodermal abnormalities. No mutation was identified in *TP63* gene. Chromosomal microarray analysis showed a 2.1 Mb deletion within chromosome region 13q31.3 in the proband and his mother; results in the other family members are pending. This deletion includes the *GPC5* gene, a highly conserved member of the glypican family of cell surface proteoglycans. The function of *GPC5* is unknown although it is expressed in a tissue-specific manner during development. *GPC5* likely plays a role in cell division as it has been shown to be amplified in follicular lymphoma and rhabdomyosarcoma; suggesting a sensitivity to copy number changes. To our knowledge, there have been no previous reports of individuals with mutations or deletions of this gene. Given the features of ectodermal dysplasia plus microcephaly and intellectual disability this may comprise a new syndrome.

Pathological studies and therapeutic testing of isofagomine in a viable mouse model of neuronopathic Gaucher disease. *Y. Sun[1], K. Kitatani[4], H. Ran[1], B. Liou[1], M. Zamzow[1], D. P. Witte[3], M. R. Skelton[2], M. T. Williams[2], C. V. Vorhees[2], Y. A. Hannun[4], G. A. Grabowski[1]* 1) Div Human Genetics, CCHMC, Cincinnati, OH; 2) Div of Neurology, CCHMC, Cincinnati, OH; 3) Div of Ped Pathology, CCHMC, Cincinnati, OH; 4) Dept of Biochemistry and Mol Biology, Med U of SC, Charleston, SC.

Gaucher disease is the most common lysosomal storage disease caused by defective acid -glucosidase (GCase) function. To develop a model for therapeutic studies in Gaucher disease, we generated mice of subacute neuronopathic type Gaucher disease by crossing the point mutated GCase (V394L/V394L) mice with saposin C deficient mice (C-/-). Saposin C is a lysosomal protein needed for optimal GCase activity. The resultant mice (4L;C*) began to exhibit CNS abnormalities at ~30 days. Death occurred ~48 days due to neurological deficits. Axonal degeneration was evident in brain stem, spinal cord and white matter of cerebellum accompanied by increasing infiltration of CD68 positive microglial cells and activation of astrocytes in the brain stem, basal ganglion, thalamus and hindbrain regions. Electron microscopy showed inclusion bodies in neuronal processes. Relative to V394L/V394L mice, 4L;C* mice had diminished GCase protein and activity. Marked increases (20-30 fold) of glucosylsphingosine (GS) and moderate elevation (1.5-3 fold) of glucosylceramide (GC) were detected in the brain. In contrast to the CNS, increase of GC and GS in the liver and lung did not develop storage cells. Neuronal cells in the hippocampus from 4L;C* mice had significantly attenuated long-term potentiation than WT mice. The 4L;C* mice mimic the CNS phenotype and biochemistry of some type 3, neuronopathic variants of Gaucher disease. The treatment of isofagomine tartrate, a selective chaperone for GCase, in 4L;C* mice increased GCase activity by 1.6-5.3 fold. Importantly, the treated mice showed delayed onset and significant extension of their life span by ~25%. Those results demonstrated that 4L;C* mouse is a unique and viable model suitable for testing chaperone and substrate reduction therapies, and investigating the mechanisms for neuronopathic Gaucher disease.

A variance components method to test for QTL heterogeneity among multiple ethnic groups. *T. Kippola[1], K. Edwards[2], S. Santorico[3]* 1) Department of Statistics, Oklahoma State University, Stillwater, OK; 2) Department of Epidemiology and the Institute for Public Health Genetics, School of Public Health and Community Medicine, University of Washington , Seattle, Washington; 3) Department of Mathematical and Statistical Sciences, University of Colorado, Denver, Colorado.

Findings from the Third National Health and Nutrition Examination Survey suggest that as many as 1 in 4 Americans may be affected by metabolic syndrome (Ford et al. 2002). As with many diseases, the prevalence of metabolic syndrome varies greatly among ethnic groups (Park et al. 2003). We wish to simultaneously investigate the etiology of metabolic syndrome among multiple ethnic groups using quantitative trait linkage methods. Numerous methods exist for conducting linkage analysis for a quantitative trait locus (QTL); however, these methods do not allow for heterogeneity that may arise when testing for linkage using multiple families from different ethnic groups, countries or geographic locations. We propose a method, which is an extension of the variance components method suggested by Almasy and Blangero (1998). Our method can determine if there exists statistically significant linkage while allowing for heterogeneity among different ethnic groups, countries or geographic locations. We also propose a test for determining if heterogeneity exists. Our method can incorporate group specific non-genetic differences via covariates and varying levels of polygenic and environmental variability prior to assessing whether the variability due to potentially common QTLs is the same. Additionally our method allows for each group to use a different marker map. Both tests are based on likelihood ratio test statistics and will be applied to an existing data set consisting of genotype and metabolic syndrome phenotype data for 69 Caucasian, 53 Mexican-American, 65 African-American and 15 Japanese-American extended kindreds. Simulation results of various statistical properties, such as power and Type I error rate, of our proposed method will be presented.

Novel Enzyme Replacement Therapy for Gaucher Disease: On Going Phase III Clinical Trial with Recombinant Human Glucocerebrosidase Expressed in Plant Cells. *D. Aviezer[1], E. Almon-Brill[1], Y. Shaaltiel[1], G. Galili[4], R. Chertkoff[1], S. Hashmueli[1], E. Galun[3], A. Zimran[2]* 1) Protalix Biotherapeutics, Karmiel, Israel; 2) Gaucher Clinic, Shaare Zedek Medical Center, Jerusalem, IsraeL; 3) Goldyne Savad Institute of Gene Therapy, Hadassah Hebrew University Hospital, Jerusalem,Israel; 4) Plant Sciences, Weizmann Institute of Science, Rehovot, Israel.

Gaucher Disease, characterized by glucocerebrosidase (hGCD) deficiency, provokes glucosylceramide accumulation in cellular lysosomes. Disease clinical pathology includes anemia, thrombocytopenia splenomegaly, skeletal pathology and pulmonary hypertension/infiltration. Current therapy uses mammalian based production of recombinant glucocerebrosidase for enzyme replacement therapy (ERT) that involves post-expression glycan remodeling for exposing mannose structures, required for intake by Macrophages. Protalix has developed a propriety plant cell expressed active form of rh-glucocerebrosidase (prGCD). The unique protalix technology permits control of glycosylation pattern and consistency through targeting to specific plant cell organelles. Hence, prGCD has intrinsic exposed mannose residues and demonstrates batch to batch consistency. prGCD exhibits similar crystal structure and biological activity to that of the currently used CHO expressed Cerezyme using in-vitro assays. Preclinical toxicology studies showed no treatment-related adverse events, no neutralizing antibodies and no clinical findings. Phase I safety clinical trial showed that prGCD administered intravenously in sequential doses (15, 30 and 60 units/kg) was well tolerated, all tests being within normal ranges, with no treatment related adverse events. Pharmacokinetic analysis demonstrated a prolonged half life. All immunological specific tests were within normal ranges. An international multi-center Phase III Pivotal trial is currently ongoing under FDA Special Protocol Assessment approval where 30 untreated patients will be administered with 30U/kg or 60U/kg per infusion over a period of 9 months. Following completion of the protocol, patients are offered to enter an Extension study. In addition, a switch-over study to prGCD is to begin soon.

Amplification of the BP1 homeobox gene in breast cancer. *L. Cavalli[1], Y.-G. Man[2], A. Schwartz[3], I. Cavalli[4], P. Berg[5], B. Haddad[1]* 1) Georgetown University, Lombardi Comprehensive Cancer Center, LL S165A, Washington, DC; 2) Armed Forces Institute of Pathology, Department of Gynecologic and Breast Pathology, Washington DC; 3) George Washington University Medical Center, Dept. of Pathology, Washington, DC; 4) Federal University of Parana, Department of Genetics, Curitiba, PR, Brazil; 5) George Washington University Medical Center, Dept. of Biochemistry and Molecular Biology, Washington, D.C.

The BP1 gene, mapped to 17q21.33, is a homeobox gene that has been implicated in breast tumorigenesis. Studies have shown BP1 mRNA overexpression in 80% of infiltrating ductal carcinoma and its correlation with cancer progression and invasion. A central question is the mechanism(s) of activation of the BP1 gene these tumors. Our main objective was to assess the copy number status of the BP1 gene in primary breast tumors (PBT) and sentinel lymph node (SLN) metastasis to determine whether BP1 protein expression is caused by gene amplification. Formalin-fixed, paraffin-embedded sections of 36 breast cancer lesions, including 12 cases of PBT and 12 pairs of PBT with corresponding SLN metastasis, were analyzed by Fluorescent in situ Hybridization (FISH) using a BP1 probe. In 20 of these cases FISH analysis was also performed for the HER2/NEU gene (localized at 17q21). The results were correlated with immunohistochemistry (IHC) data for BP1 and HER2/NEU proteins. Increased BP1 copy number was observed in 33% of the cases, with a frequency of 36% and 29% in the PBT and SLN metastasis, respectively. BP1 protein was expressed in 91% of the samples: in all of the PBT with increased BP1 copy number and 78.6% of PBT with normal copy number. HER2/NEU amplification was detected in 22% of the cases. Concordance between BP1 and HER2/NEU copy numbers was found in 68% of the PBT and 90% of the SLN metastasis We have shown that the BP1 homeobox gene is amplified in primary and metastatic breast tumors, with a significant correlation with HER2/NEU amplification. Considering that BP1 expression was observed in cases with both increased and normal BP1 copy number, we conclude that other mechanisms in addition to gene amplification play a role in BP1 protein expression.

A new phenotype in the Stickler/Marshall continuum: COL11A1 mutations and growth deficiency in childhood.

L. Sloper[1], B. F. Griswold[1], R. M. Liberfarb[2], C. A. Francomano[3], M. Mannikko[4], L. Ala-Kokko[4], N. B. McDonnell[1] 1) Clinical Research Branch, NIA/NIH, Baltimore, MD; 2) Massachusetts General Hospital, Boston, MA; 3) Greater Baltimore Medical Center, Towson, MD; 4) University of Oolu, Finland.

Stickler Syndrome is an autosomal dominant disorder caused by mutations in the collagen genes including COL2A1, COL11A1, and COL11A2. Manifestations include facial dysmorphisms, cleft palate, vitreoretinal abnormalities, hearing loss, spondyloepiphyseal dysplasia, joint laxity and premature osteoarthritis. COL11A1 mutations are also implicated in Marshall syndrome, in which additional features include frontal bossing, short upturned nose, persistence of flat facies into adulthood, and relatively shorter final stature. We have recently identified three probands born with cleft palate, hearing loss and high myopia, with splice site mutations in COL11A1, who presented with an additional complication of growth deficiency in childhood despite adequate nutritional intake. All patients (age range 3-9) were pre-pubertal children with height <3 centile; with delayed bone age, low IGF-1, and were being considered for growth hormone replacement. Additional features noted included frontal bossing, short nose, and large eyes. Brain MRI of one of the children showed a short clivus and basilar invagination of the odontoid process. We analyzed final heights and growth curves of patients with Stickler Syndrome with known mutations who were seen at the NIH. It was noted amongst eight probands and family members with COL2A1 mutations, the final stature was consistently above average. Three probands with COL11A1 mutations, now adults, were noted to have final stature in the 5-15 centile with no history of growth deficiency. This cohort of three young patients suggests that there is a newly defined phenotype that includes growth deficiency in the Stickler/Marshall continuum associated with COL11A1 mutations. The availability of growth hormone treatment represents a new challenge in the management of this syndrome. Given a general lack of success with other skeletal dysplasias, it is difficult to endorse such an intervention at this time.

Identification of susceptibility genes for complex diseases using pooling-based genome-wide association scans. *Y. Bosse[1], F. Bacot[2], A. Montpetit[2], J. Rung[2], H. Q. Qu[3], C. Polychronakos[3], T. J. Hudson[4], P. Froguel[5], R. Sladek[2,3], M. Desrosiers[3,6]* 1) Laval University, Quebec, PQ, Canada; 2) McGill University and Genome Quebec Innovation Center, Montreal, PQ, Canada; 3) McGill University, Montreal, PQ, Canada; 4) The Ontario Institute for Cancer Research, Toronto, ON, Canada; 5) CNRS 8090-Institute of Biology, Pasteur Institute, Lille, France; 6) Montreal University, Montreal, PQ, Canada.

The success of whole genome association studies (GWAS) to identify risk loci of complex diseases is now well-established. One persistent major hurdle is the cost of these studies. Performing GWAS on pools of DNA samples may be a cost-effective alternative. In this study, we performed pooling-based GWAS with more than 550,000 SNPs in two case-control cohorts consisting of patients with Type II diabetes (T2DM) and with chronic rhinosinusitis (CRS). In the T2DM cohort, the results of the pooling experiment were compared to individual genotypes obtained from a previously published GWAS. SNPs associated with T2DM by traditional GWAS were among the top ranked SNPs in the pooling experiment. For example, two of the five susceptibility genes identified in the original publication (TCF7L2 and HHEX) had SNPs among the top 10 allelotyping results (top 10 out of 555,175 SNPs). In the CRS cohort, the top 1536 SNPs were validated by individual genotyping. Forty-one percent of SNPs (598 out of the 1457 SNPs that passed quality control) were associated with CRS at a nominal p value of 0.05, confirming the potential of pooling-based GWAS to identify SNPs that differ in allele frequencies between two groups of subjects. These results demonstrate that the pooling experiment on high-density genotyping arrays can produce a list of top ranked SNPs that is overrepresented by true genetic associations. It also suggests that a pooling approach combined with selection of a limited number of SNPs for individual validation can substantially lower the cost of GWAS that are conventionally too expensive for diseases with limited access for funding. The low cost associated with a pooling-based GWAS clearly justifies its use in screening for major genetic determinants of complex diseases.

A Novel Base-Pair Mutation of the CSPG2 Gene in a Family with Wagner Syndrome. *E. Burner[1,2], S. Ronan[1], K. Tran-Viet[2], C. Toth[1], T. Young[1,2]* 1) Duke University Eye Center, Durham, NC; 2) Center for Human Genetics, Durham, NC.

Purpose: Wagner syndrome (OMIM 143200) is an autosomal dominant vitreoretinopathy characterized by an optically empty vitreous cavity with fibrillary condensations, a preretinal avascular membrane, retinal perivascular sheathing, chorioretinal dystrophy, lattice degeneration, and high myopia, with a predisposition to retinal detachment and cataracts. Wagner syndrome has significant phenotypic overlap with other conditions, such as ocular Stickler syndrome type 1 (OMIM 609508). Stickler syndrome type 1 maps to chromosome 12q13.11-13.2, with associated COL2A1 gene mutations. Wagner syndrome maps to chromosome 5q13-q14 (WGN1 locus), with previously reported mutations in the CSPG2 gene confirming that this is a distinct syndrome. We report a three-generation Caucasian family with 6 affected individuals clinically diagnosed with Wagner syndrome, and screening for sequence variants in the COL2A1 and CSPG2 genes. Methods: Genomic DNA samples derived from venous blood were collected from 9 total family members. Complete sequencing of COL2A1 was performed on the proband. Direct sequencing of CSPG2 was performed on all family member samples. Primers for PCR and sequencing were designed to cover all exons and intron-exon boundaries. Results: No detectable COL2A1 mutations were noted, making the diagnosis of ocular Stickler syndrome highly unlikely for this family. A unique base-pair substitution (GT) in intron 8 cosegregating with disease status was identified. This mutation occurs in a highly conserved previously reported splice site with a similar base-pair substitution (GA). Direct sequencing of this splice site mutation in 107 unrelated external controls revealed no variants, supporting the rarity of this base-pair change and its causation in Wagner syndrome. Conclusions: CSPG2 encodes versican, a proteoglycan and component of human vitreous. This novel base-pair substitution is thought to cause deletion of exon 8 and formation of a truncated protein product as previously reported. Further mutation screening of CSPG2 in additional Wagner syndrome families is important for functional characterization.

Role of X-inactivation in females with heterozygous mutations of *ARX*. *J. Sudi[1], G. Mancini[2], A. Toutain[3], M. I. Van Allen[4], S. L. Christian[1], W. B. Dobyns[1]* 1) Dept. Human Genetics, Univ Chicago, Chicago, IL; 2) Dept. Clinical Genetics, Erasmus Univ Medical Center, Rotterdam, The Netherlands; 3) Service de Génétique et Service de Neuropédiatrie, Centre Hospitalier Universitaire de Tours, Tours, France; 4) Dept. Medical Genetics, Univ British Columbia, Vancouver, B.C, Canada.

Mutations in *ARX* have been associated with X-linked lissencephaly with abnormal genitalia, infantile spasms, and less severe phenotypes in males. We have recently shown that a majority of females with severe *ARX* mutations (protein truncations or missense mutations in the homeobox) have an abnormal phenotype, excluding mothers of affected males to remove ascertainment bias (Sudi *et al*., ASHG 2007). To explain the phenotypic variability among heterozygous females, we developed a novel X-inactivation assay using a polymorphic marker (AFM260YE5) located 1.4Mb upstream of *ARX*, that contains a restriction site for the methylation-sensitive enzyme, *Aci*I. We performed X-inactivation (Xi) analysis in 21 heterozygous females including 3 probands, 13 mothers of males with XLAG, and 5 other female relatives. Overall, we detected skewing of Xi greater than 80:20 in only 1/21 females (4.7%), which is similar to the studies of control females using the *AR* assay (Amos-Landgraf *et al*., 2006) suggesting that skewing of X-inactivation is not the major cause of disease expression in heterozygous females. We next evaluated four families with two or three heterozygous females informative at the AFM locus to examine whether variations in direction of Xi less than 80:20 (mild skewing) could impact the phenotype. Here we assume that no crossovers occurred in the 1.4 Mb between the Xi locus and the *ARX* gene. In two families, the affected and normal females had mild skewing in opposite directions, while in two others the affected and normal females had mild skewing in the same direction. These results do not support a major role for mild skewing of Xi in producing the *ARX* female phenotype, although mild skewing could still be a modifying factor in some individuals. Our data suggests that other genetic factors contribute to phenotypic variability in females.

Clinical implementation of a high-resolution oligonucleotide based array-CGH platform for the detection of deletions and duplication in Dystrophin (*DMD*) and neighboring genes on Xp21. *D. del Gaudio[1], J. A. Lee[1], E. S. Schmitt[1], H. Pham[1], E. Spiegel[2], R. J. Wapner[2], L. Groome[3], C. M. Eng[1]* 1) Dept.of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas, USA; 2) Dept. of OB/GYN, Columbia University Medical Center, New York, NY, USA; 3) Dept. of OB/GYN, LSU Health Sciences Center, Shreveport, LA, USA.

Duchenne and Becker muscular dystrophies (DMD/BMD) are X-linked recessive dystrophinopathies caused by mutations in the dystrophin gene (*DMD*). Approximately 70% of mutations are intragenic deletions or duplications. We report our experience with the clinical implementation of a high-resolution oligonucleotide array-CGH platform for the identification of copy-number changes in the *DMD* gene. The analysis was performed on a total of 940 samples submitted to our clinical laboratory from August 2007 to May 2008. The initial 302 samples were analyzed on version 1.0 (4X44K) of the array, and the subsequent 638 cases were analyzed on version 2.0 (8X15K) with expanded coverage in the *DMD* exons and neighboring genes on Xp21. Array-CGH detected *DMD* copy-number changes in 277 cases. Of these, 223 were deletions, 48 duplications, and 6 complex rearrangements. One case with a 191-bp deletion within exon 19 was missed on the version 1.0 array but was subsequently correctly identified on the version 2.0 array. The ability of array-CGH analysis to clarify unique clinical scenarios is illustrated by the detection of two complex rearrangements involving *DMD* and neighboring genes on Xp21: an interrupted duplication involving *DMD* exons 58 through 60 and the entire *DAX1* gene distal to *DMD* in a male fetus, and an unusual rearrangement composed of a deletion of *DMD* exons 1 through 7 and a duplication of the *XK* and *CYBB* genes identified in a male fetus and in his carrier mother. Our results highlight the utility of high-resolution array-CGH in detecting copy-number changes in the *DMD* gene in affected males and carrier females. In addition, this analysis allows improved identification and interpretation of complex rearrangements involving *DMD* and the neighboring genes on Xp21 maximizing genotype-phenotype correlations and improving patient counseling.

Sex-specific gene flow between Pygmy and non-Pygmy populations. *T. S. Simonson[1], W. S. Watkins[1], Y. Zhang[1], M. J. Bamshad[2], L. B. Jorde[1]* 1) Department of Human Genetics, University of Utah, Salt Lake City, UT; 2) Department of Genome Sciences, University of Washington, Seattle, WA.

Cultural traditions and preferences may drive sex-specific gene flow among human populations. We have examined sex-specific gene flow between Mbuti Pygmies, a hunter-gather population, and surrounding agriculturist groups, the Alur, Hema, and Nande, which all reside in Central Africa. We used 18 lineage-defining Y chromosome SNPs and HVS1 mitochondrial DNA sequence information to examine patterns of gene flow among these groups. Mbuti Pygmy males have more diverse Y chromosome lineages (Mbuti Pygmy [n = 28]: = 0.229; Alur [n = 10]: 0.193; Hema [n = 18]: 0.178; Nande [n = 15]: 0.090) and slightly less mtDNA diversity than neighboring groups (0.020, 0.023, 0.025, 0.022 in Mbuti Pygmy, Alur, Hema, and Nande groups, respectively). The majority of Mbuti Pygmy males have a Y haplotype characteristic of Mbuti Pygmies (B2b); however, more than 30% of Pygmy males exhibit Y haplotypes associated with Bantu-speaking agricultural populations (E3a lineage). Conversely, no agriculturist males exhibit Y haplogroups associated with Mbuti Pygmy populations but instead have derived Y haplogroups characteristic of Bantu agriculturalists (E2, E3a). Pairwise F_{ST} was calculated among all populations using Y haplogroup frequency and HVS1 mtDNA sequence data. YDNA and mtDNA F_{ST} values between Mbuti Pygmy and non-Pygmy groups (Alur, Hema, and Nande) were 0.278, 0.355, and 0.217 (for YDNA) and 0.088, 0.239 and 0.217 (for mtDNA), respectively. A Mantel test between pairwise F_{ST} matrices showed no significant correlation ((r = 0.27; p 0.35), which indicates that patterns of genetic differentiation differ between Y chromosome SNPs and mtDNA sequence patterns. These results also suggest no emigration of Mbuti Pygmy Y chromosomes into surrounding groups but immigration of non-Mbuti Pygmy Y chromosomes into the Mbuti Pygmy population.

Control of population stratification by correlation-selected principal components. *S. Lee[1], F. Zou[1,2,3], F. A. Wright[1,2,3]* 1) Departments of Biostatistics, University of North Carolina, Chapel Hill, NC; 2) Carolina Center for Genome Sciences, University of North Carolina at Chapel Hill, NC; 3) Center for Environmental Bioinformatics, University of North Carolina at Chapel Hill, NC.

In genome-wide association studies, population stratification is recognized as inflating Type I error by producing overdispersion of test statistics. Principal component-based methods applied to genotypes provide information about population structure, but the precise relationship between principal components and the over-dispersion of test statistics has not been established. We have identified a relationship between principal components and over-dispersion of association test statistics that provides precise guidance on the selection of principal components to use in adjusted test statistics. While methods such as Eigenstrat use the eigenvalues alone to determine which principal components to employ, in our approach the principal components are selected based on both eigenvalues and the relationship of the principal components to the disease phenotype. Our analytic results provide a direct connection among genomic control methods, principal component methods, and more recent approaches which attempt to use the relationship between markers and disease phenotype in obtaining adjusted test statistics. For real data analysis, our approach selects a much smaller number of principal components than that suggested by Tracy-Widom statistics, providing substantial computational savings in genome scans.

Toward the delineation of the clinical diagnostic criteria for the early onset seizure variant of Rett syndrome. *M. Maria Antonietta[1], R. Artuso[1], R. Polli[2], S. Sartori[2], F. Ariani[1], M. Pollazzon[1], A. Marozza[1], M. R. Cilio[3], F. Vigevano[3], M. Vecchi[2], C. Boniver[2], B. Dalla Bernardina[4], A. Parmeggiani[5], S. Buoni[6], J. Hayek[6], F. Mari[1], A. Renieri[1], A. Murgia[2]* 1) Medical Genetics, University of Siena; 2) Pediatric Neurology Unit, University of Padua; 3) Division of Neurology, Bambino Gesù Childrens Hospital, Rome; 4) Servizio Neuropsichiatria Infantile, Università di Verona; 5) Child Neurology and Psychiatry Unit, University of Bologna; 6) Child Neuropsychiatry, Azienda Ospedaliera Senese, Siena, Italy.

We report a detailed clinical investigation of 9 girls with CDKL5 mutations to delineate the specific clinical diagnostic criteria of the Hanefeld variant of Rett syndrome. Four subjects were previously reported (Scala et al. J Med Genet 2005; Mari et al. Hum Mol Genet 2005) and 5 are new cases (p.R178W, p.Q347X, p.N71D, pV132G, p.E203D). Patients range from 14 months to 9 years. All present epilepsy with variable onset from 10 days to 3 months. Girls experience different seizures at onset and during the whole course of the disease; multiple seizure types may occur as well. After the antiepileptic treatment patients may have a short free period but epilepsy progressively relapses. Patients do not show a classic regression period due to the very early onset of the seizures. Even if an apparently normal perinatal period is referred, the girls were described as irritable, easy to cry, drowsy and poor sucking. Typical stereotypic hand movements severely affecting ability to grasp are showed. Psychomotor development is severely impaired. In all girls the head circumference was and is within the normal range, indicating that head growth should be considered among minor diagnostic criteria. Each patient was classified using a severity score through the evaluation of 22 different clinical signs (Renieri et al Brain Dev in press). Results were compared with 128 classic and 25 PSV MECP2-mutated patients, all evaluated by the same clinical geneticists. Both the statistical and the descriptive approach have been used to delineate clinical diagnostic criteria for the Hanefeld variant.

Safety and tolerability of miglustat (Zavesca) in patients with infantile onset GM2 gangliosidosis. *C. Tifft[1], S. Yang[1], C. Morgan[1], A. Vanderver[2], T. Lateef[2], B. Moorjani[2], P. Glass[2], B. Brooks[3]* 1) Div Genetics & Metabolism, Children's Natl Medical Ctr, Washington, DC; 2) Center for Neuroscience & Behavioral Medicine, Children's Natl Medical Ctr, Washington, DC; 3) Opthalmic Genetics Branch,National Eye Institute, NIH, Bethesda, MD.

Miglustat, a competitive inhibitor of the first step in ganglioside synthesis, has been shown to decrease GM2 storage and improve clinical outcome in Sandhoff disease mice. The drug is approved for the treatment of Gaucher disease in adults, but experience with miglustat in very young children is limited. In this open-label study 6 patients with infantile GM2 gangliosidosis were treated with miglustat for 26 weeks at an equivalent adult dose of 200 mg/kg/day. Pharmacokinetics were assessed after a single dose and at steady state at weeks 1 and 13 respectively. Side effects were monitored and disease progression assessed using EEG, evoked response (VER, BAER), developmental and neurological evaluations, and biomarkers in cerebrospinal fluid (CSF) including chitotriosidase, CCL18, and GM2 quantitation by tandem mass spectrometry.

Our results show that miglustat is well-tolerated with fewer gastrointestinal side effects than adult patients. At baseline, GM2 ganglioside was markedly elevated in patient vs. control CSF and did not change significantly over the 26-week treatment period. Chitotriosidase, a measure of activated macrophage/microglial cell burden, was 54-fold elevated ($p<0.001$) and macrophage marker CCL18 was 4-fold elevated over control CSF ($p<0.01$). There was no significant change in these biomarkers during the study. All patients showed variable rates of clinical decline. Evoked response testing suggests that vision declines more rapidly than hearing. Interestingly, the Sandhoff disease patient showed near complete clearance of PAS positive inclusions in conjunctival cells. We conclude that although well-tolerated, miglustat cannot prevent the relentless decline in patients with infantile GM2 gangliosidosis. However, we have identified several surrogate biomarkers and clinical tools that may be useful in monitoring disease progression in the future.

The ribosomal biogenesis protein EMG1 is mutated in Bowen-Conradi Syndrome. *J. Armistead[1], S. Khatkar[1], B. Meyer[2], P. Koetter[2], E. Nylen[1], S. Liu[1], G. Coghlan[1], K. Wrogemann[1], C. Greenberg[1], KD. Entian[2], T. Zelinski[1], B. Triggs-Raine[1]* 1) Biochemistry and Medical Genetics, University of Manitoba, Winnipeg, Manitoba, Canada; 2) Center of Excellence, Macromolecular Complexes, Institute of Molecular Biosciences, Johann Wolfgang Goethe-University, Frankfurt, Germany.

Bowen-Conradi syndrome (BCS) is an autosomal recessive disorder characterized by severely impaired prenatal and postnatal growth, profound psychomotor retardation, and death in early childhood. BCS is of major concern to the Hutterites of the Canadian Prairies and the United States Great Plains because of its morbidity, mortality, and high birth prevalence (1/355). We previously mapped the BCS gene to a 1.9 Mbp interval on human chromosome 12p13.3 between *F8VWF* and *D12S397*. The 59 genes in this interval were ranked as candidates for BCS and 36 of the best candidates were sequenced. Numerous variants were identified, but only an A to G mutation causing an Asp to Gly substitution in the 18S ribosome assembly protein EMG1 was a good candidate to cause BCS. This mutation segregated with disease, was not found in 207 non-Hutterite controls, and affected an amino acid conserved in all identified homologues. *EMG1* mRNA was found in most adult tissues and its levels did not differ between BCS patient and control fibroblasts. To investigate the effect of the mutation on the EMG1 protein, vectors encoding HA-tagged wild type and mutant EMG1 were constructed and expressed in mammalian cells. The level of detectable soluble mutant EMG1 was significantly reduced compared to the wild type protein. Metabolic labeling with ^{35}S-Cys/Met showed that the Asp to Gly mutation did not reduce the rate of EMG1 synthesis, but instead led to its sequestration in a detergent-insoluble compartment. Fluorescence microscopy in mammalian cells did not differentiate the sub-cellular localization of mutant and wild type EMG1. In yeast, EMG1, also known as NEP1, is an essential gene and EMG1 harbouring the Asp to Gly mutation interfered with RNA binding as well as dimerization and cellular localization of the protein. These findings strongly support our conclusion that the Asp to Gly mutation disrupts the normal function of EMG1 resulting in BCS.

Regulation of Direct-to-Consumer Genetic Tests. *L. Geddes, T. Caulfield, R. Hyde-Lay* Health Law Institute, Law Centre, University of Alberta, Edmonton AB, T6G 2H5.

The completion of the Human Genome Project has given rise to a plethora of genetic tests for everything from cancer to personalized diet plans. Although these tests seem to offer a new age of personalized medicine, some are concerned that it is too soon to be offering these tests, especially directly to the public. Internet companies have been springing up rapidly, offering these tests directly to the consumer and often without the involvement of a physician or genetic counselor. Regulation of these tests can be difficult; the Internet spans national and state boundaries, attempts to restrict advertising may be challenged by freedom of speech jurisprudence, and the distribution of authority to regulate tests is often unclear. For example, in the United States the FDA has jurisdiction over the pre-market review of genetic tests only when they are sold as a test kit as opposed to tests that are developed in house by clinical laboratories and marketed as a service. The majority of genetic tests available are developed in house and are therefore not subject to pre-market review. Similar uncertainty exists in Canada, where in-house testing services do not seem to be captured by existing legislation covering medical devices. This abstract provides an overview of recommendations for regulation being made in the literature as well as by the media. Preliminary observations show that recommendations frequently include the involvement of a trained professional in the testing process, the regulation of information provided in advertisements and on company websites, and the provision of educational resources to medical professionals and consumers. Further, media portrayals of genetic tests show a mix of caution and excitement, and often recommend that the consumer be cautious when purchasing a genetic test. The availability of genetic tests directly to consumers creates an interesting regulatory problem for policy makers. An overview of these problems and existing recommendations may help paint a clearer picture of what regulations are currently needed.

Genome-wide Association Analysis of Multiple-Drug Severe Adverse Events. *Y. Shen[1], B. Jagla[1], A. Floratos[1], M. Nelson[3], S. John[4], E. Lai[3], Q. Li[5], A. Califano[1], A. Holden[6], I. Pe'er[1,2]* 1) Center for Computational Biology and Bioinformatics, Columbia University, New York, NY; 2) Department of Computer Science, Columbia University; 3) Pharmacogenetics, GlaxoSmithKline, NC; 4) Pfizer Inc; 5) Johnson and Johnson; 6) Severe Adverse Effects Consortium, LTD.

Severe adverse events (SAEs) due to drug treatment are usually rare but acute, potentially life threatening occurrences that not only pose a serious public health concern, but also can limit or even eliminate the use of otherwise beneficial agents. Pharmacogenetics seeks to identify strong risk alleles for SAEs, facilitating a molecular-level understanding of biological mechanisms and potentially develop predictive tests to focus safety concerns on carriers. In contrast to common diseases, there is expectation of large effects. The International SAE Consortium (SAEC) set out to systematically scan for genetic SAE risk factors by pulling together samples and resources from more than a dozen pharmaceutical, government, and academic partners, with aggressive sharing policy of raw data and analysis results (http://www.saeconsortium.org). We present the first genome-wide association study by the SAEC focused on serious skin rash, which includes stevens-Johnson syndrome and toxic epidermal necrolysis, widespread burn-like reactions to a variety of drugs that can be lethal. We genotyped 72 cases and 135 matched controls with the Illumina 1M chip, a study powered to identify large genetic risk factors with potential predictive utility. We further improved the power of this study by inclusion of up to 1934 additional control samples from publicly available datasets. We detail methodological demography and quality control pitfalls in this procedure of aggregating controls, along with approaches to overcome such issues. Our results highlight several genomic regions that may play a role in SSR risk for several drugs, including confirmation of the HLA locus. Furthermore, these results support the absence of highly penetrant common genetic risk factors that could be responsible for most SSR events. SAEC is committed to continue and tackle this and other adverse events, with several projects already being planned and structured.

Detecting rare variants for complex traits using linkage and association analysis. *X. Zhu, T. Feng, Q. Lu, R. Elston*
Epidemiology & Biostatistics, Case Western Reserve Univ, Cleveland, OH.

Large genome-wide association studies have been evident to detect common genetic variants involved in common diseases. However most of the variants account for only a small portion of a trait variance. Candidate gene based resequencing approach also suggests that many rare genetic variants contribute to a trait variance. Here we proposed two designs: sibpair and unrelated case designs to detect rare genetic variants in candidate gene based analysis or genome-wide association. We show first that we are able to classify the rare risk haplotypes together using a small portion of sample and have power to test association in the remaining sample. This method can be straightforwardly applied to resequencing data. We next applied the method to WTCCC hypertension data, which is the only trait no genome-wide association evidence was reported in the original study, and identified one interesting gene associated with hypertension at genome-wide significance level. These results suggest that searching rare genetic variants is feasible and can fruitful in current genome-wide association studies, candidate gene studies or resequencing studies.

Clinical implementation of a high-resolution oligonucleotide based array-CGH platform for the detection of deletions and duplications of *MECP2* and neighboring genes on Xq28. *J. Lee, P. Fang, C. Eng, D. del Gaudio*
Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Mutations in *MECP2* cause Rett syndrome, a progressive neurodevelopmental disorder that affects ~1:10,000 females. *MECP2* mutations have been detected in females and males with a wide spectrum of neurodevelopmental phenotypes. Diagnostic sequencing of the *MECP2* coding region detects ~86% of mutations in classic Rett syndrome. We previously implemented dosage-sensitive Southern analysis to identify copy-number changes in *MECP2*, which account for an additional ~10% of cases. To augment the detection of these rearrangements, particularly heterozygous duplications in females, we had developed a real-time PCR assay and also utilized MLPA for confirmation by a second methodology. In an effort to explore new technologies that would enable higher resolution copy-number analysis of *MECP2* and the surrounding genes, we recently designed an oligonucleotide comparative genomic hybridization array (array-CGH). This array includes ~700 oligonucleotide probes covering *MECP2* (introns and exons) with an average spacing of ~1 probe per 100 bp; this version of the array also contains probes covering the neighboring genes at a similar density. On this platform we tested 7 blinded control samples (6 females, 1 male) that were previously analyzed by Southern and MLPA analyses and showed a spectrum of rearrangement sizes. Our array-CGH assay correctly detected *MECP2* partial gene deletions in 3 females (exon 1 deletion, exons 1 and 2 deletion, and exon 4 partial deletion), whole gene duplications in 1 female and 1 male that extended beyond the *MECP2* gene, and no copy-number change in 1 female. For one female patient, an exon 3 partial deletion (c.205_c.377+287del460) was detected by a single probe loss. Our data demonstrate the potential utility of array-CGH analysis in detecting *MECP2* rearrangements, particularly duplications in females and rearrangements extending beyond *MECP2*, with high sensitivity, and highlight the value of covering the entire *MECP2* gene, the neighboring genes, and flanking intergenic sequences to delineate most accurately these rearrangements in a clinical diagnostic setting.

Performance of genome-wide association studies for the identification of genes with known genetic association with asthma. *A. J. Rogers[1], B. A. Raby[1], J. A. Lasky-Su[1], A. Murphy[1], R. Lazarus[1], C. Lange[2], E. K. Silverman[1], S. T. Weiss[1]* 1) Channing Laboratory, Brigham & Women's Hosp, Boston, MA; 2) Harvard School of Public Health, Boston MA.

Rationale: GWAS studies promise to detect common disease susceptibility genetic variation. However, it is unclear whether this study design will overtake candidate gene based studies given the burden of correction for multiple comparisons imposed by this design and the inter-locus variability of coverage of common variation. Using genotype data from a GWAS in asthma, we assessed whether we could detect associations to replicated asthma-susceptibility candidates. **Methods**: GWAS was performed in 422 white asthmatic children and their parents, using the Illumina HumanHap 550v3 array. We identified replicated (2 populations) asthma-associated candidate genes through review of published literature. We performed family-based association testing assuming an additive genetic model using FBAT. **Results**: Review of literature identified 42 genes (163 SNP) with evidence of reproducible association with asthma. The sample size of our cohort was larger than >90% of studies, suggesting adequate power to detect marginal associations for the majority of loci. Genotype data was available for 892 SNP mapping within 50kb of these loci, including 31 SNPs previously associated with asthma. In our dataset, SNP associations ($p<0.05$) were observed for 63 SNP in 15 of the candidate genes, including 3 of 5 positional candidates (PHF11, GPR-154, and DPP10). The most significant associations were with DPP10 ($p=0.0008$). None of the observed associations were significant after correction for multiple comparisons. Importantly, we did not observe association for the 31 SNPs previously associated with asthma. **Conclusions**: The majority of SNP previously associated with asthma were poorly represented in this study. While we showed marginal replication of several of the genetic loci, SNP-level replication was not achieved. Despite the value of GWAS in identifying novel biology, their role in assessing replicated candidate genes remains questionable. U01 HL075419, U01 HL65899, P01 HL083069, R01 HL 086601, and T32 HL07427.

Mechanisms of JAG1 missense mutations in Alagille Syndrome and Cardiac Disease. *R. C. Bauer[1,2], A. O. Laney[1], E. Goldmuntz[1,2], N. B. Spinner[1,2]* 1) Children's Hospital of Philadelphia, Philadelphia, PA; 2) University of Pennsylvania, Philadelphia, PA.

Mutations in JAG1, a ligand in the Notch signaling pathway, cause autosomal dominant Alagille Syndrome (AGS), which is characterized by liver, cardiac, skeletal and ocular symptoms. JAG1 mutations have also been reported in individuals with apparently isolated cardiac disease. The cause of this variable expressivity is not completely understood. Over 45 unique missense mutations have been identified in AGS patients and an additional 4 mutations have been reported in patients with isolated cardiac disease. We analyzed 12 AGS missense mutations distributed throughout the various domains of the JAG1 protein as well as the 4 cardiac mutations. We assessed subcellular localization, post-translational modification and ability to signal via the Notch receptors. We found 3 classes of mutations. In the first class are 8 mutations that cause improper glycosylation and improper trafficking (C78S, C92Y, Y181N, C229Y, C234Y, C693Y, P810L, C902S). These mutant proteins are retained intracellularly and therefore cannot interact with the Notch receptors. The second class contains 4 mutations that are leaky in that 2 species of proteins are observed: one that is trafficked to the cell surface and one that is retained intracellularly (C271R, G274D, C284F, C438F). The protein that does make it to the cell surface still cannot activate the Notch signaling pathway. The third class of mutants is also leaky, however, in this group the proteins present at the cell surface are able signal through the Notch receptor (C664S, C714Y, C740R, C911Y). The clustering of mutations in classes 2 and 3 suggests clues for functional characteristics of specific protein regions. Examples of mutations that fit into each of these classes have been identified in both patients with classic features of AGS and in patients with apparently isolated cardiac defects. This suggests that the variable expressivity seen in these patients is not caused by intrinsic properties of the mutations, as previously thought, but rather by additional modifying factors, which could be genetic, environmental or stochastic.

Combined genomewide linkage and association study of ocular refraction in the Framingham Eye Study. *R. Wojciechowski[1], D. Stambolian[2], L. D. Atwood[3], J. E. Bailey-Wilson[1]* 1) IDRB, NHGRI, Baltimore, MD; 2) Ophthalmology & Genetics, Un of Pennsylvania, Philadelphia, PA; 3) Dept of Biostatistics, Boston University, Boston, MA.

Purpose: Refractive disorders are the most common causes of visual impairment worldwide. Previous studies have reported linkage of myopia or ocular refraction to a number of loci, but no genomewide association results have been published. We report results of genomewide linkage and association scans in Framingham Eye Study (FES) families. Methods: Eye exams were conducted on 2,540 FES participants in 293 families. We performed quantitative trait linkage and association analyses on ocular refraction, defined as the spherical equivalent refractive error. Genotypes were available for 1,240 individuals at ~113,000 SNP markers. Variance-components (VC) linkage analysis was performed with the Merlin package using a subset of ~18,000 SNPs. Family-based association statistics were estimated using FBAT and Merlin-assoc. FBAT was performed under additive genetic models for single SNPs and haplotypes using a 3-SNP sliding window. Results: VC linkage analyses yielded a peak LOD score of 4.16 (p=0.00001) at 124 cM on chr. 2q14.3. The strongest association in the FBAT haplotype analysis was found in the chr. 2 linkage region (p=0.0009 at ~80.96 Mb or 103 cM) . Merlin-assoc statistics yielded the strongest evidence of association with rs1049467 (p=8.2 x 10e-7; chr1q31.2, ~190Mb or 210 cM). There was suggestive evidence of linkage to that region (LOD=1.42, p=0.005 at 232cM). Conclusions: We found significant linkage of ocular refraction to a region on chr.2q. Though not genomewide significant after Bonferroni correction, association results are consistent with the presence of loci influencing ocular refraction on chromosomes 1 and 2. Nearby genes have not previously been reported as functional candidates for ocular phenotypes. Further investigation of these regions with a denser SNP map is warranted.

Founder mutations in the *PMS2* gene. *L. Senter-Jamieson[1], M. Clendenning[1], S. Sun[2], J. Panescu[1], S. Gallinger[3], J. Mackay[4], J. Larsen Haidle[5], M. Greenblatt[6], J. Young[7], A. de la Chapelle[1]* 1) Human Cancer Genetics Program, Ohio State Univ, Columbus, OH; 2) Mathematical Biosciences Institute, Ohio State Univ, Columbus, OH; 3) Mt Sinai Hosp, Toronto, ON, CA; 4) Dept of Biology, Univ College London, UK; 5) Univ of Iowa Hosp and Clinics, Iowa City, IA; 6) Univ of Vermont, Burlington, VT; 7) Queensland Inst of Med Res, Brisbane, AUS.

Germline mutations in the mismatch repair (MMR) gene, *PMS2,* are associated with Lynch syndrome. We recently published a series of *PMS2* gene mutations, 11 of which occurred in multiple unrelated probands. We previously characterized one such mutation in exon 7 (c.736_741del6ins11) that occurred in 12 unrelated probands. A shared ancestral haplotype was found, indicating that this is a prevalent mutation with reduced penetrance. Likewise, a missense mutation (c.137GT; S46I) was observed in 7 unrelated probands of European descent. Six probands had colorectal cancer and 1 had cancer of the small intestine. All tumors demonstrated absence of PMS2 only by IHC. Haplotype analysis was possible in 5 probands, and DNA from informative relatives was obtained for 4 of the families. All available individuals were typed at 6 microsatellite and 10 SNP loci, which span the *PMS2* region. In 3 of these families, a common haplotype was found which was shared by 2 affected individuals in each family, but not by unaffecteds. This haplotype spans ~98.5-356Kb and was not observed among 96 control haplotypes. In the fourth family, a smaller haplotype was shared. We also identified an exon 10 deletion in 3 unrelated probands from Australia, and confirmed the same breakpoints (c.989-296_144+706del1158) as two cases reported by others. No haplotype data have been obtained from these individuals to date given paucity available of DNA. Both the c.137GT mutation and the exon 10 deletion in *PMS2* likely represent additional founder mutations in the *PMS2* gene while founder mutations in *MLH1*, *MSH2*, and *MSH6* are less common. This could be due, in part, to the reduced penetrance of *PMS2*-associated disease in comparison to the other MMR genes and could explain why more than 20 cases of biallelic *PMS2* mutation carriers have been reported in the literature.

Toward Delineation of the clinical Syndrome associated with the 17q21.31 Microdeletion. *J. Leroy[1], F. Faes[2], B. Loeys[1], R. Van Coster[2], F. Speleman[1], A. De Paepe[1], A. Gijsbers[3], C. Ruivenkamp[3], E. De Baere[1], B. Menten[1]* 1) Dept Med Genetics, Ghent Univ Medical Sch, Ghent, Belgium; 2) Division of Pediatric neurology, Dept Pediatr, Ghent Univ Medical Sch, Ghent, Belgium; 3) LUMC, The Netherlands.

Recently the recurrent microdeletion 17q21.31 was identified in some individuals with syndromic mental retardation by use of array comparative genomic hybridization. The detection of this deletion in three independently ascertained mental retardation patients prompted attention to their mutual clinical similarity. Our patients were born at or slightly post term in 1990 (Pt.1, female), 1999 (Pt. 2, male) and 2006 (Pt. 3, male) respectively following an uncomplicated pregnancy and vertex delivery in Pts 2 and 3. Because of fetal bradycardia, emergency caesarian section was required in Pt 1. Anthropometric data at birth were within normal limits and have been throughout. All had significant motor and slow cognitive development, but no signs of regression. Hypotonia has remained significant throughout childhood in Pts 1 and 2, but is milder in Pt 3, who achieved sitting unaided at 9 months, whereas this milestone was not reached before 15 months in the former two. Walking started at 20 months in the boys, but not until 28 months in the girl. Although onset occurred after age two, speech development progressed well subsequently in all. Dysmorphic facial features included prominent, tall forehead and oblong facial configuration, enlarged intercanthal distances, broad nose with bulky tip (not evident in Pt 3), eyelid ptosis in Pt 2, upslanting palpebral fissures and convergent squint. All had marked joint hypermobility. All belong to the mildly retarded, have a pleasant disposition and benefit effectively from special education classes. MRI of the CNS in Pt 1 revealed nearly complete absence of the corpus callosum and syringomyelia in segments D7-D10. The microdeletion 17q21.31, *de novo* in the three patients causes a recognizable clinical syndrome. Work is ongoing in order to establish the potential causal role played by the inversion polymorphism frequently observed precisely in this chromosomal region.

Gaucher Disease Diagnostic Dilemma: When the Molecular Analysis and Clinical Presentation Disagree - The N370S/L444P Allele. *M. Balwani, M. E. Grace, R. J. Desnick* Department of Genetics and Genomic Sciences, Mount Sinai School of Medicine, New York, NY.

The diagnosis of Type I Gaucher Disease (GD) was made by prenatal carrier screening of the acid -glucosidase (GBA) gene in a 31 year old Ashkenazi Jewish (AJ) woman who had the common mild N370S allele and the more severe L444P mutation. GD patients with N370S/L444P typically manifest symptoms at a mean age of 16.9 years, with mean splenic and hepatic volumes of ~22x and 2x normal, and early-onset bone involvement (Sibille et al 1993). Follow-up evaluation at our Center found the proband to be essentially asymptomatic: a normal physical examination, no organomegaly, and normal blood counts (Hgb=13.3 g/dL, WBC=8.2x 103/cm3, Platelets=344,000/cm3). Leukocyte GBA activity was in the low normal range (2.9 U/mg, nl: 1.6 - 8.5 U/mg). Clinical laboratory studies and plasma biomarkers for GD were normal, including chitotriosidase (20 U/ml, nl: 300U/ml). The patient declined MRI studies; however a skeletal survey and bone density were normal. To investigate this quandary, cDNAs from this individual, obtained by RT-PCR amplification, were sequenced. Of the 10 cDNAs sequenced, 50% were negative for the N370S and L444P alleles. The remaining cDNAs carried both mutations. Thus, this individual was heterozygous for GD, having a very rare allele with the two most common GD mutations. Family studies revealed that the p.[N370S; L444P] allele was inherited from her 63 year old asymptomatic mother who had normal leukocyte GBA activity (2.0 U/mg) and plasma chitotriosidase (72 U/ml). The N370S allele is present at a frequency of ~ 1 in 17 in AJ individuals, and occurs in ~70% of AJ GD patients, while the L444P occurs infrequently (<3%) in AJ GD patients (Grabowski, 1997). Thus, the mechanisms for the p.[N370S; L444P] allele include crossing-over, or gene conversion with the pseudogene, or less likely, a second independent mutation (Beutler et al 2000). This unique mutation emphasizes the limitations of only molecular studies for the diagnosis of this disease.

Using Exon Skipping to Rescue Common Mutations in Hermansky-Pudlak Syndrome Type 1. *L. M. Vincent, R. Hess, W. Westbroek, W. A. Gahl, M. Huizing* MGB, NHGRI/NIH, Bethesda, MD.

Hermansky-Pudlak syndrome (HPS) is characterized by oculocutaneous albinism, a bleeding diathesis, and other systemic complications, including granulomatous colitis and fatal pulmonary fibrosis, due to defects in intracellular vesicle trafficking. The most common subtype, HPS-1, occurs primarily among Puerto-Ricans and results from a 16-bp duplication in exon 15 of the *HPS1* gene. Little is known about the function and structure of *HPS1*, making directed therapy difficult. In view of successful advances in therapeutic exon skipping (e.g., Duchenne Muscular Dystrophy), we utilized anti-sense morpholino oligonucleotides (MOs) to induce in-frame exon skipping of exons carrying deleterious *HPS1* mutations, specifically those in exons 12, 13, 15, and 16. Normal melanocytes positively transfected with MOs were selected using FACS analysis and observed over time. We utilized RT-PCR analysis to validate the effect of each MO on the *HPS1* transcript and immunofluroescence (IF) staining against the melanosome-specific protein TYRP1 to detect abnormal melanosomal trafficking patterns (i.e. lack of accumulation in dendritic tips). Exon 12 MO, ille12, effectively removed exon 12 in ~75% of *HPS1* transcripts. IF analysis revealed a decrease in the accumulation of melanosomes at the dendritic tips; however, it is unknown if this was due to partial activity of the mutant HPS1 protein or residual normal HPS1. Exon 13 MO, e13i13, removed exon 13 in ~95% of *HPS1* transcripts and likewise showed reduced, but not abolished, trafficking of melanosomes to the dendritic tips. We are currently administering these corresponding mutation-specific MOs to patient melanocytes in order to determine if we can rescue the HPS-1 phenotype. Interestingly, co-transfection of i11e12 and e13i13 removed both exon 12 and exon 13 in 100% of HPS1 transcripts and trafficking of melanosomes to the dendritic tips was abolished suggesting the resultant mutant product lacks HPS1 function. Further investigation into targeted exon skipping could result in clinical applications for the treatment of the fatal pulmonary fibrosis associated with HPS-1.

Linkage analysis of candidate genes in Carribean Hispanic families affected with Dementia with Lewy Bodies. *C. Reitz[1,2], J. Lee[1,2,3,6], A. Nervi[1], E. Katz[1], R. Mayeux[1,2,4,6], L. N. Clark[2,5,6]* 1) G.H. Sergievsky Center, Columbia University, New York, NY; 2) Taub Institute for Alzheimer Disease and Aging Research; 3) Department of Epidemiology; 4) Department of Neurology; 5) Department of Pathology; 6) Center for Human Genetics.

Background: Mutations in several Parkinson and Alzheimer disease genes including alpha-Synuclein, beta- Synuclein, Leucine Rich Repeat Kinase 2, Glucocerebrosidase, Presenilin 1 and Presenilin 2 have been identified in patients with dementia with Lewy bodies (DLB). Although the majority of patients with DLB are thought to be sporadic, families have also been described suggesting a genetic component. Objective: To explore chromosomal regions harboring DLB candidate genes in nine multiplex Caribbean Hispanic families with multiple members affected with DLB using 264 microsatellite markers (average intermarker distance of 9.3 cM). Results: Significant LOD scores >1.0 suggestive for genetic linkage were obtained for several markers and in several chromosomal regions. Chromosomal regions harboring known DLB genes and related pathways in addition to other chromosomal regions with significant LOD scores were further analyzed. One such region of interest was 1q21.3. At an average intermarker distance of 9.3 cM, marker D1S1679 which maps to region 1q21.3 and is 7cM apart from Glucocerebrosidase (GBA) at 1q22, showed significant genetic linkage with the DLB phenotype (LOD=0.87, p= 0.03). Conclusions: Here we describe the results of linkage analysis for exploration of DLB candidate genes in nine Caribbean Hispanic families with multiple family members affected with DLB. Several chromosomal regions including, locus 1q21.3 which is close to the chromosomal region harboring GBA, were significantly linked with the DLB phenotype. Our findings highlight these regions for further genetic exploration in DLB research.

Characterization of UGTs active against Suberoylanilide Hydroxamic Acid (SAHA). Variations in SAHA glucuronidation associated with UGT genetic variants. *R. M. Balliet, G. Chen, R. W. Dellinger, C. J. Gallagher, D. Sun, P. Lazarus* Penn State University College of Medicine, Hershey, PA.

SAHA is a histone deacetylase inhibitor that has been FDA approved to treat cutaneous T-cell lymphoma and is currently being used in 47 clinical trials to treat various cancers. A major mode of SAHA metabolism is via glucuronidation. The goal of the present study was to characterize the SAHA glucuronidation pathway and identify genetic factors within this pathway that could play a role in overall response to SAHA. As determined by ultra-pressure liquid chromatography and confirmed by sensitivity to treatment with -glucuronidase and LC-MS, UGTs 1A1, 1A3, 1A4, 1A6, 1A7, 1A8, 1A9, 1A10, 2B7 and 2B17 all exhibited glucuronidation activity against SAHA *in vitro*, with the hepatic UGT2B17 and the extra-hepatic UGTs 1A8 and 1A10 exhibiting the highest overall V_{max}/K_Ms after normalization for UGT protein expression by Western blotting (16, 33 and 24 $nL.min^{-1}.g$ over-expressed UGT $protein^{-1}$, respectively). The $UGT1A8^{173Gly277Cys}$ variant exhibited a significant ($P<0.03$) 3-fold decrease in glucuronidation activity as compared to wild-type UGT1A8; the $UGT1A8^{173Ala277Tyr}$ and $UGT1A10^{139Lys}$ variants exhibited no glucuronidation activity against SAHA *in vitro*. HLM from male subjects who were homozygous for the UGT2B17 whole-gene deletion exhibited a significant ($P<0.001$) 50% decrease in glucuronidation activity as compared to HLM from individuals with at least one UGT2B17 allele; no association between UGT2B17 genotype and HLM glucuronidation phenotype was observed in females. This gender-specific association between UGT2B17 genotype and glucuronidation activity was correlated ($R^2 = 0.30$) with UGT2B17 expression in the same liver specimens as determined by real-time PCR. These results suggest that several UGTs may play an important role in the metabolism of SAHA and that the presence of one or more variant UTGs could significantly alter SAHA clearance rates and potentially play an important role on overall patient response to SAHA. These data further suggest that there are gender-specific differences in hepatic UGT2B17 expression and activity that may affect drug clearance.

Morphometric Analysis of the Posterior Fossa and Spinal Cord in patients with Hereditary Disorders of Connective Tissue. *J. C. Quo[1,2], L. Sloper[1], R. Raza[2], N. B. McDonnell[1]* 1) Clinical Research Branch, NIA, NIH, Baltimore, MD; 2) Harbor Hospital Center, Baltimore, MD.

Patients with hereditary disorders of connective tissue (HDCT) have increased incidence of Chiari I malformation, however, abnormalities of the posterior fossa or the spinal cord has not been systematically evaluated to date. We retrospectively evaluated midline sagittal brain, midline sagittal lumbar spine, and axial lumbar magnetic resonance imaging (MRI) of 94 subjects with HDCT (70 Female, 24 Male, aged 12-71) and 209 age matched controls (138 Female, 71 Males). HDCT subjects included those with diagnoses of Ehlers-Danlos syndrome (EDS), Marfan syndrome, and Fibromuscular Dysplasia with connective tissue features (FMD). The MRI images were obtained on a 1.5 Tesla MRI machine (Philips Interna, USA) with system 11.1 software. The measurement that showed significant differences between the subjects and the controls included the basiocciput measurement ($p=0.0115$), foramen magnum distance ($p=0.0101$), opisthion to internal occipital protuberance (IOP) ($p=0.0188$), dorsa sella to IOP ($p=0.0176$), tonsillar herniation ($p<0.0001$), fourth ventricle height ($p=0.0020$), brainstem length to the craniocervical junction ($p<0.0001$), brainstem length to the inferior portion of the gracile tubercle ($p=0.0016$), tentorium slope ($p=0.0109$), tentorium slope to the twining line ($p=0.0044$), height of cerebellum ($p<0.0001$), conus medullaris level ($p=0.0383$), and filum terminale thickness ($p<0.0001$). Measurements that were not statistically significant included the basisphenoid length, ponto-medullary height, medullary height, and odontoid angle. The significant p value observed in 13 of the 17 measurements suggests that there are consistent morphometric differences that are seen in HDCT patients that can contribute to increased incidence of clinical abnormalities such as Chiari I malformation, tethered cord syndrome and related complaints.

Identification of TP53 mutations in Mexican Li Fraumeni families. *S. Vidal[1], L. Taja-Chayeb[1], O. Gutierrez-Hernandez[1], A. Duenas-Gonzalez[1,2]* 1) Dept Clinical Research, Inst Nacional de Cancerol, Mexico City, Mexico; 2) Unidad de Investigación Biomédica en Cáncer.Instituto de Investigaciones Biomédicas, UNAM/Instituto Nacional de Cancerología.

Li Fraumeni syndrome (LFS) is a rare disease characterized by early tumor onset, multiple tumors in individuals and multiple affected family members; most cases (70%) are associated with dominant germline mutations in the tumor suppressor TP53. There are no known germline mutations at TP53 for the Mexican mestizo population. The purposes of this work were to determine the mutation frequency of TP53 gene, and to analyse what kind of mutations are present in our LFS patients. This study was carried on through denaturing high performance liquid chromatography (DHPLC) analysis. Any abnormal DHPLC profile that suggested a mutation was corroborated by direct sequencing. Those families with mutations will be follow up for early detection, and will receive genetic counseling. 15 LFS patients were included; DNA was obtained from peripheral leukocytes, and was amplified for the coding regions as well as the adjacent intronic sequences. 7 pairs of primers were used to analyse exons 2 to 11. Of the 15 families studied, we found deleterious mutations in 4, two missense mutations in exon 5 and 8, one splicing mutation that affect exon 5 and results in a stop codon (187), and one novel insertion of seven nucleotides in codons 329-330 which has never been reported. Besides, several polymorphisms were detected. Until now, 26.7% of our population has mutation in TP53 gene. Mutation negative families are currently being studied with different sets of primers to corroborate the absence of mutations.

Mouse Mutants as Models for Human Developmental Malformations: The *Extra-Toes Spotting (Xs)* Mouse. *D. Gildea[1,2], S. Loftus[1], Y. Yang[1], W. Pavan[1], L. Biesecker[1]* 1) GDRB, NIH/NHGRI, Bethesda, MD; 2) Genetics, GWU, Washington, DC.

Greig cephalopolysyndactyly syndrome (GCPS) is a malformation syndrome that includes limb anomalies, specifically polydactyly and syndactyly. GCPS is caused by mutations in *Glioma-associated oncogene-3 (GLI3)*, which is part of Sonic hedgehog (SHH) signaling. The GLI3/SHH pathway regulates many developmental processes, including limb patterning. Dysregulation of this pathway due to mutations in *GLI3* can result in limb malformation. The Extra-toes ($Gli3^{Xt}$) mouse is an animal model for GCPS. Like the human phenotype, $Gli3^{Xt}$ mice exhibit preaxial polydactyly. Another mouse, Extra-toes spotting (*Xs*), shares a similar phenotype with $Gli3^{Xt}$. *Xs* mice exhibit preaxial polydactyly, coat hypopigmentation, limb length asymmetry, and microphthalmia. Previous mapping excluded mouse *Gli3* as the gene mutated in Xs^J (Jackson allele) mice, and the gene and Xs^J mutation remain unknown. To identify the gene, we are performing recombination mapping in Xs^J mice. We maintain our Xs^J mice on a B6C3FeF1/J background, where penetrance of the phenotype is 82%. For mapping, it was necessary to outbreed Xs^J mice to castaneus mice to introduce a distinct chromosomal background, as we encountered substantial homozygosity in the candidate interval. Offspring from this outcross do not exhibit the Xs^J phenotype. When breeding offspring from the castaneus outcross to B6C3FeF1/J mice, we experienced a penetrance of 39%. These data show variable penetrance of the Xs^J phenotype that is dependent upon mouse genetic background. Here we present our genetic analysis, phenotypic characterization, mapping data, and developmental analysis of the Xs^J animal. We mapped the Xs^J locus to a 322 kb region on mouse chromosome 7 and are evaluating candidate genes. Since the $Gli3^{Xt}$ and *Xs* phenotypes overlap, we hypothesize that the gene mutated in Xs^J mice is a gene in the Gli3/Shh pathway. To test this hypothesis, we used *in situ* hybridization to evaluate in Xs^J embryos the expression of genes in the Shh/Gli3 pathway. Data show misexpression of Shh members in Xs^J embryos, suggesting that the gene mutated in Xs^J mice is in the Shh pathway.

Searching for rare mutations in autism spectrum disorders, from SLC6A4 to novel candidate genes. *T. Sakurai, G. Cai, E. Hoffman, J. D. Buxbaum* Dept Psychiatry, Mount Sinai Sch Medicine, New York, NY.

It has long been recognized that autism can result from inherited and de novo chromosomal abnormalities and single gene mutations. However, for any given chromosomal abnormality or single gene mutation, the prevalence in individuals with autism is typically below 1 percent. With the development of methods of molecular cytogenetics and high-throughput sequencing, the capacity to identify such variants has improved markedly. We have undertaken deep re-sequencing of candidate genes as a means of identifying previously unidentified causes of autism. To do this we have evaluate several screening methods (including methods such as high-resolution melting curve analysis and additional novel methods). One such method was previously applied to a cohort of ca. 800 individuals with nearly equal numbers of cases with autism spectrum disorders and controls, to evaluate the role of rare variants in the 2q37 region in autism. We examined more than 1000 amplicons. Using this same method, we have examined the candidate gene SLC6A4 in the same cohort. We have begun sequencing genes that fall within regions of recurrent deletions in autism, including such regions on chromosomes 2 and 16. We sequence 300-400 unrelated subjects with an autism spectrum diagnosis (giving us > 95% power to detect a variant occurring in 1 percent of cases) and typically search for de novo mutations as the most compelling evidence for a causal relationship with the disorder. To date we have found no de novo mutations in brain-expressed candidate genes in the 2q or 16p region, but additional genes are now being evaluated. Our data demonstrate that rare variants in SLC6A4 are not associated with autism or with specific behaviors within autism (as defined by factors within the ADI-R).

Combination of acid -glucosidase D409H and saposin C deficiency in mice leads to visceral glucosylceramide accumulation. *G. A. Grabowski[1], K. Kitatani[2], S. Barnes[1], H. Ran[1], J. Brandewie[1], Y. A. Hannun[2], Y. Sun[1]* 1) Division of Human Genetics, Cincinnati Children's Hospital Medical Center and Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, OH; 2) Department of Biochemistry and Molecular Biology, Medical University of South Carolina, Charleston, SC.

The defective lysosomal hydrolysis of glucosylceramide (GC) in Gaucher disease is caused by mutations in the gene encoding acid -glucosidase (GCase). Saposin C is a lysosomal protein having unique activation and proteolytic protective functions for GCase. To develop an additional model of Gaucher disease and to explore the in vivo effects of saposin C deficiency on GCase function, the point mutated GCase (D409H/D409H) mice were backcrossed to saposin C deficiency (C-/-) mice. The resultant mice, 9H;C*, developed visceral storage cells in the liver and lung that were identified as macrophages by positive CD68 staining. 9H;C* mice showed similar GCase activity as D409H/D409H mice. LC/MS analysis revealed 4-10 fold increased GC levels in 9H;C* liver and lung while brain GC levels remained at wild type levels. Life span, neurological phenotype, and CNS pathology in 9H*C mice were not distinct from C-/- mice. In contrast to 4L;C* mice (V394L/V394L;C-/-), a neuronopathic Gaucher disease model, 9H;C* mice presented primarily visceral involvement of GC accumulation. These models provide insights into potential interaction of saposin C with GCase mutations leading to a tissue-specific disease phenotype. This mouse model will be useful for evaluating therapeutic intervention and understanding the interaction of saposin C and GCase in vivo, and will greatly contribute to advancing our knowledge of glycosphingolipid storage diseases.

Treatment With Enzyme Replacement Therapy in a Severely Affected Individual With Hunter Syndrome (MPS II). *N. Arciniegas[1], A. S. Cornier[2], C. Burgos[2], N. Ramirez[3], J. Flynn[3], S. Carlo[2,4]* 1) La Concepcion Hospital, Dept. of Pediatrics, San German, PR; 2) La Concepcion Hospital, Molecular Medicine Dept., San German, PR; 3) La Concepcion Hospital, Dept. of Orthopaedics, San German, PR; 4) Ponce School of Medicine, Dept. of Biochemistry, Ponce, PR.

Hunter Syndrome (MPS II) is caused by the absence of the enzyme iduronate sulfatase.The deficiency of this enzyme gives rise to lysosomal accumulation of heparan and dermatan sulfate. The accumulation of these mucopolysaccharides in the connective tissue affects multiple systems including the musculoskeletal, cardiovascular, pulmonary and ocular systems. It is the only type of mucopolysaccharidosis with X-linked recessive transmission. We are presenting the case of a 16 year old male patients with MPS II , who was on enzyme replacement therapy (ERT) for the last 12 months of his life. The patient was evaluated before enzyme replacement therapy. The evaluation included physical examination, cell blood count, urine GAGS levels and abdominal sonogram. Patient had monthly follow up visits for evaluation. Weekly intravenous enzyme infusion was performed using the recommended doses for age and weight. Initial physical examination and determination of urinary levels of GAGS were elevated, abdominal sonogram presented hepatomegaly, brain magnetic resonance reported communicating hydrocephaly and cell blood count showed thrombocytopenia. After nine months of treatment patient had normal levels of urinary GAGS and hepatomegaly had disappeared, platelet count was within normal limits. At physical examination the patient displayed the typical dysmorphic features of MPS II, purulent nasal secretions and limitation of range of movement of the joints, manifestations have improved considerably after the treatment, including allergies and sinusitis. Patient died of complications of the disease after one year of treatment. ERT is beneficial for short and long term treatment of patients with lysosomal storage diseases. Majority of symptoms improve with the treatment making the quality of life of patient and family members improve considerably.

A High-Resolution Smaller Insertion and Deletion Map of a Human Genome by Next-Generation, High-Throughput Paired-End Sequencing. *E. F. Tsung[1], H. E. Peckham[1], Z. Zhang[2], S. S. Ranade[2], C. C. Lee[1], C. R. Clouser[1], J. M. Manning[1], C. L. Hendrickson[1], L. Zhang[1], E. T. Dimalanta[1], T. D. Sokolsky[1], J. K. Ichikawa[1], F. C. Hyland[2], J. M. Kidd[3], C. Alkan[3], J. A. Malek[4], F. M. De La Vega[2], G. L. Costa[1], E. E. Eichler[3], K. J. McKernan[1]* 1) Applied Biosystems, Beverly, MA, 01915; 2) Applied Biosystems, Foster City, CA 94404; 3) Dept. of Genome Sciences, HHMI, University of Washington, Seattle, WA; 4) Weill Cornell Medical College in Qatar, Doha, Qatar.

Hybridization microarrays and fosmid-end sequencing reveal that structural variants (SVs) including insertions and deletions are common and extensive. Microarray methods, however, lack resolution and are blind to unbalanced events, while clone-based end-sequencing is time consuming and expensive. Here, we present a high-resolution survey of insertion and deletion SVs from a human genome, a HapMap Yoruba sample (NA18507), by ultra-high throughput sequencing of paired-end libraries with the Applied Biosystems SOLiD System. We have sequenced a variety of 2x25-bp paired-end libraries with insert sizes ranging from 600bp to 3.5kb (SD 10-23%) as well as several longer read length paired-end libraries. The paired-end libraries provide over 178x physical (clone) coverage and over 6x sequence coverage from uniquely placeable mate pairs derived from unique molecules.

The variety of insert sizes and the high physical coverage reached delivers an unprecedented level of resolution which allows the detection of very small indels within single tags (1 to 11 bp), as well as larger indels in the range of 20 bp to over 100 kb, thus closing the gap of the detectable indel sizes. Approximately 90,000 small indels including 24,000 one base pair insertions and 22,000 one base pair deletions have been detected with high specificity. Also, the sensitivity approaches fosmid-sequencing in regions of high sequence coverage. Overall, our results provide a comprehensive assessment of the requirements to discover insertion and deletion variants in large-scale studies of genetic variation in human populations and human diseases with next-generation sequencing.

PHYLO-GENETICS VARIATION IN EUROPEAN PERCIDS. *A. Islam[1], O. Gorshkov[2], V. Chernov[2], V. Kuznetsov[3]* 1) Dept Ophthalmology, Schepens Eye Res Inst, Harvard University Medical School, Boston, MA 02114, USA; 2) Kazan Institute of Biochemistry and Biophysics, Russian Academy of Sciences, Kazan, Russia 420111; 3) Dept Vertebrate Zoology, Kazan State University, Kazan, Russia 420008.

The genetics variation of Percids of Russian Kuibyshev lake evolved compact phylo-evolutionary relationship bond among the populations. Pikeperches (*Stizostedion lucioperca* and *S. volgense*), river perch (*Perca fluviatilis*) and ruffe (*Gymnocephalus cernuus*) are the most ancient species of this reservoir, which have the same external appearances with different sizes and shapes. The declining tendency of pikeperches in this reservoir urges us to investigate their genetics relationship. The genetics variation and rapidly amplified DNA polymorphism between the exploited fish populations, a product of past evolution in relation to the ecology is an essential initiative to increase the overall chances that depleted population will regain and persist over the long period of time. Two microsatellites DNA from walleye (*S. vitreum*) were used as primers for this study. Each of the DNA markers (Svi-4 and Svi-18) resolved to 4-6 alleles with a size range of 102-124 bp. The DNA analysis revealed diversified polymorphism in different Percid populations. The PCR products of *S. volgense* have amplification of 100-1200 bp with rapidly amplified polymorphic DNAs. Most of the dominant DNA bands of pikeperches existed in the size range of 100-600 bp in both of the primers treatments. *S. lucioperca* had diversified DNA amplifications with a size range of 100-400 bp where 2-3 dominant DNA bands were located at 100-250 bp. River perch had only one band at 100 bp and ruffe had two DNA amplification signals at 100 and 180 bp. The rapidly amplified DNA polymorphism of different Percids showed a specific size range of the DNA amplifications. *S. lucioperca* and *S. volgense* revealed as closely related species. The phylo-evolutionary genetics variation of these species established that over-fishing, geographical, temporal and reproductive isolations are the primary barriers that produce changes in populations and genetics compositions. These analyses could help us further to understand the human phylo-genetics variation as well.

Chromosome 9p21 Polymorphisms Confer Risk of Myocardial Infarction in the INTERHEART Study. *R. Do[1], C. Xie[2], J. Kimberly Haladyn[2], S. D. Bailey[1], A. Belisle[3], A. Montpetit[3], S. Yusuf[2], J. C. Engert[1], S. S. Anand[2] on behalf of the INTERHEART Genetics Investigators* 1) Department of Human Genetics, McGill University, Montreal, QC; 2) Population Health Research Institute, McMaster University, Hamilton, ON; 3) McGill University and Genome Quebec Innovation Center, McGill University, Montreal, QC.

Genome-wide association (GWA) studies have identified genetic variants in the chromosome 9p21 locus that influence the risk of coronary heart disease (CHD)/myocardial infarction (MI) and type 2 diabetes (T2D) in several European populations. To date, the findings for CHD/MI have been studied in two non-European populations, with replication found in East Asians but not African Americans. In the present study, we investigated the effect of chromosome 9p21 variants on MI in 1,867 South Asians from the INTERHEART study. Six SNPs identified from GWA studies of CHD/MI and T2D were selected. Minor allele frequencies (MAF) for the T2D SNPs, rs564398 and rs10811661, were 0.25 and 0.15 respectively, while MI SNPs (rs10757274, rs2383206, rs10757278, rs1333049) ranged from 0.43 to 0.47. The T2D SNP, rs10811661, was in low linkage disequilibrium (LD) with all other SNPs ($0 < D < 0.11$). There was strong LD between all four MI SNPs ($D > 0.95$), with decreasing LD between MI SNPs and the other T2D SNP (rs564398; $0.73 < D < 0.78$). After adjusting for age and sex, five of the six SNPs were associated with MI (all $p<0.01$), with the strongest result being rs10757274 ($p=0.0004$). In addition, the most common haplotype (MAF=0.49) was associated with an increased incidence of MI ($p=0.0004$). Conditional haplotype tests that adjusted for the nine risk factors for MI shown previously in the INTERHEART study (diabetes, apoB/apoA1, waist/hip, current smoking, hypertension, alcohol consumption, fruit and vegetable consumption, psychosocial factors and physical activity) yielded the strongest result ($p=0.00005$). These results suggest that chromosome 9p21 variants are a strong risk factor for MI in South Asians and this effect is independent of nine risk factors for MI. Further assessment of these variants is required in other ethnicities.

Tethered Spinal Cord in Patients with Vascular Ehlers-Danlos Syndrome. *S. Bangura[1], B. F. Griswold[2], L. Sloper[2], R. Raza[2], N. B. McDonnell[3]* 1) Laboratory of Clinical Investigation, National Institute on Aging, NIH, Baltimore, MD; 2) Clinical Research Branch, National Institute on Aging, NIH, Baltimore, MD; 3) Harbor Hospital, National Institute on Aging, Intramural Research Program, NIH, Baltimore, MD.

Tethered Spinal Cord Syndrome (TSC) is a progressive neurological disorder caused by fatty tissue attachment that limits the movement of the spinal cord within the spinal column, causing an abnormal stretching of the spinal cord. Symptomatology includes urinary and bowel dysfunction, lower extremity pain, numbness or weakness, scoliosis, foot deformities and abnormal reflexes. Here we report two female patients (age 14 and 43) out of 16 consecutive probands with the vascular form of Ehlers Danlos Syndrome (VEDS)who were discovered to have TSC by Magnetic Resonance Imaging (MRI) of the lumbar spine. Patient #1 (Age 14) had a glycine substitution mutation, c.3563 G>A (G1021E) and had excessive bruising, abnormal scarring and wound healing problems, but no vascular events to date. Patient #2 had a 108 bp deletion in COL3A1 (exons 26 and 27) and had vascular complications including rupture of the abdominal aorta and multiple aneurysms in the splenic artery. Patient #2's MRI revealed open spina bifida and tethered cord at the level of L5. She had previous surgery for bladder incontinence, and currently suffers from urinary retention, which are not known complications of VEDS and likely related to TSC. Patient #1 had spinal cord tethered at the level of L3. She has chronic constipation, which is often seen in VEDS patients, but can also be a manifestation of TSC. Both patients had lower extremity weakness and pain. Physical findings included scoliosis, genu vera and pes planus, which can be seen in both TSC and VEDS. Patient #1 had abnormal lower extremity reflexes. This study describes the first reported case series of TCS in the setting of VEDS. Some findings, such as the lower extremity and spine deformities can be caused by both TSC and VEDS. A lumber spine MRI is indicated in the setting of VEDS if suspicious signs and symptoms of TSC are present.

Fine Mapping of a Myopia Susceptibility Locus on Chromosome 22q12. *D. Stambolian[1], R. Spielman[2], G. Ibay[3], K. Gogolin Ewens[2], R. Wojciechowski[3], J. Bailey-Wilson[3]* 1) Dept Ophthamology, Univ Pennsylvania, Philadelphia, PA; 2) Dept. Genetics, Univ Pennsylvania, Philadelphia, Pa; 3) NHGRI, NIH, Baltimore, MD.

Myopia is an increasingly common disease caused by an inability to focus images on the retina. Currently, there are 14 known loci but no genes are known to cause myopia. In an effort to delineate the genetic susceptibility to myopia, we have previously performed a genome-wide screen in a Jewish population of 56 families ascertained by a proband with myopia. The genomic region with the strongest evidence for linkage with myopia was located on chromosome 22q12 (HLOD=4.73). We have recently constructed a high-density SNP map of this 35 Mb region in the Jewish population, utilizing LD data available from the International HapMap Project. A total of 1386 SNPs were genotyped with minor allele frequencies of >0.10 in this Jewish population. Eleven SNPs were not informative in the analysis. Association analyses were performed with common myopia (-1D or worse) in extended pedigrees using FBAT. Nine SNPs clustered around 30 Mb had p-values <0.001. Most of these SNPS were positioned in the intergenic regions of PISD, SFI1 and EIFENIF1. One SNP remained statistically significant after adjustment for multiple testing using the Bonferroni correction and is located in the 3 UTR of PISD. Additional work is in progress to replicate and confirm these findings in independent multi-ethnic populations and to determine the overall relevance of these SNPs in myopia susceptibility.

APOE Testing for Alzheimers Disease (AD) Risk Assessment: the Potential for Underestimating Risk. *S. Hiraki[1], J. S. Roberts[4], C. A. Chen[3], R. C. Green[1,2]* 1) Dept of Neurology, Boston Univ School of Medicine; 2) Dept of Medicine (Genet), Boston Univ School of Medicine; 3) Data Coord Center, Boston Univ School of Public Health (1-3 Boston, MA); 4) Dept of Health Behavior and Health Education, Univ of Michigan School of Public Health; Ann Arbor, MI.

Genetic test results are usually presented in binary terms while the implications of these results may be expressed as a risk. Much attention has focused on the potential negative effects of testing positive for a disease mutation while less consideration has been given to the potential for false reassurance from negative results. The REVEAL Study is a randomized controlled trial to evaluate the impact of providing a genetic risk assessment for AD on unaffected first-degree relatives of AD patients. Participants were randomized to receive APOE-based risk assessment either through a traditional education/counseling protocol or a highly condensed protocol. In this sub-study, we explored the association between APOE result (4+ vs. 4-) and underestimating risk, defined as perceiving ones risk as lower or equal to someone who does not have a family history of AD (ie. 10-15% risk). Participants (n=220) were given a lifetime AD risk estimate between 18 and 75%. Those who tested 4- (mean lifetime risk = 31%) were significantly more likely than those who were 4+ (mean lifetime risk = 50%) to underestimate their risk by rating it as the same or lower than someone without a family history of AD (35% vs. 6%, $p<0.001$). This relationship was also demonstrated in our multivariate logistic regression (OR=7.3, $p<0.001$) after controlling for age, sex, race, income, education, number of affected relatives, and randomization arm. Those testing 4- were also more likely to classify their risk as very low/low (29% vs. 7%, $p<0.001$) and to disagree with a statement of concern about developing AD (24% vs. 8%, $p=0.003$). In providing genetic risk assessment for common disease, the probabilistic nature of the information in contrast to the all-or-none result may lead to misinterpretation. Improved methods of communication of genetic susceptibility test results are needed to ensure adequate understanding of this complex information.

MCTP2 plays an important role in left ventricular outflow tract development. *S. R. Lalani[1], S. M. Ware[4], X. Wang[1], L. Potocki[1], G. Zapata[1], M. Bray[1], A. C. Chinault[1], B. A. Boggs[1], E. K. Brundage[1], J. A. Towbin[3], A. Patel[1], S. D. Fernbach[1], M. Baker[2], S. L. Hamilton[2], K. L. McBride[5], J. W. Belmont[1]* 1) Dept of Molecular and Human Genetics; 2) Dept of Molecular Physiology and Biophysics; 3) Dept of Cardiology, Baylor College of Medicine, Houston, TX; 4) Division of Molecular Cardiovascular Biology, Cincinnati Children's Hospital, Cincinnati, OH; 5) Nationwide Childrens Hospital, Columbus, OH.

Left ventricular outflow tract obstruction (LVOTO) defects have been reported in individuals with terminal deletions of 15q26. Using array-comparative genomic hybridization (CGH), we identified two half-siblings with coarctation of the aorta (CoA) and dysmorphic features, with 2.1 Mb microdeletion of 15q26.2, involving a single gene, MCTP2 (multiple C2 domain and transmembrane region protein). Their mother was found to be mosaic for this deletion which was not identified in 14,200 individuals subjected to the clinical array CGH. Targeted array CGH analysis of MCTP2 identified a de novo 40 kb duplication from intron 10 to intron 21 within the MCTP2 gene, in an individual with non syndromic CoA and hypoplastic left heart, which, if fully spliced, would predict a frameshift and premature termination at F697X. We then sequenced MCTP2 gene in 142 patients with LVOTO and found five missense variants, each one inherited from a parent and not found in 200 ethnically matched controls. To further investigate the role of MCTP2 in cardiac development, we carried out loss of function experiments by injecting Xenopus laevis embryos with a splice blocking antisense morpholino oligonucleotide. Morphant embryos underwent gastrulation and neurulation normally, however between stages 30-40 of development, a significant number of phenotypically normal appearing morphants died. At stages 44/45, MCTP2 morphants showed progressive edema with no evidence of endocardial cushion formation at any level of the developing outflow tract suggesting a possible defect in epithelial-mesenchymal transformation and confirming a role for MCTP2 in outflow tract development. These results identify MCTP2 as a novel genetic cause of coarctation of the aorta and related cardiac malformations.

Lung disease severity phenotype in Cystic Fibrosis modifier gene studies: benefits of population based samples.

C. Taylor[1], R. Dorfman[1], L. J. Strug[1], W. Li[1], A. Sandford[2], P. D. Paré[2], J. Zielenski[1], P. Durie[1], M. Corey[1] 1) Sick Kids Research Institute, Toronto, Canada; 2) James Hogg Centre, UBC, Vancouver, Canada.

Mutations in Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene cause CF but do not predict lung disease severity, the main cause of morbidity and mortality. The CF Modifier Study studies modifier genes for CF lung disease in a population based sample. Successes identifying modifier genes have been accompanied by conflicting results across study groups, hampering progress in the field. Two explanations are varying ascertainment criteria and the use of different derived phenotypes. Forced Expiratory Volume in 1 second (FEV1) predicts survival in CF and is accepted, clinically, as the best lung phenotype, generally reported as percent of predicted lung function based on age, gender and height (fev%pred). However, because of mortality selection in patients beyond early adulthood, fev%pred is a poor indicator of relative disease severity in older patients. Kulich et al., instead, used a CF specific percentile score (fevcf%; Am J Respir Crit Care Med 2004; 172:885-891) for patients under 40 years of age, which classifies severity relative to other CF patients of the same age but not across ages. Here we define a lung function phenotype for genetic analysis that can include all CF patients across age ranges and ascertainment schemes, and we compare heritability between this phenotype, fev%pred and fevcf%. We used three year longitudinal data from the CF Modifier Study, which consists of 60% of the Canadian CF population. Cohort survival estimates from the same national population were used to create a normalized survival adjusted lung phenotype (fevz). We calculated the Intraclass Correlation Coefficient (ICC) for 87 sibling pairs to get an estimate of heritability. Interestingly, the ICC for fev%pred (0.266) was similar to that for fevcf% (0.267), while the ICC was higher using fevz (0.411). Fevz is a more heritable phenotype for lung disease severity in this population. Association studies with fevz and reported modifiers will help to determine the utility of fevz for genetic analysis.

Preimplantation Genetic Diagnosis, Reprogenetics, and Policy Making: The Missing Participants in the Debate.

C. Bouffard[1], B. M. Knoppers[2] 1) Genetics Service, Faculty of Medicine and Health Sciences, Université de Sherbrooke (Quebec, Canada); 2) Canada Research Chair in Law and Medicine, Faculty of Law, Université de Montréal (Quebec, Canada).

Although preimplantation genetic diagnosis (PGD) is the first reprogenetic technique to be offered in a clinical setting, its precursor role for reprogenetics, genetics, regenerative medicine, and other fields is currently underestimated. Meanwhile, the highly colored language that pervades the debate around PGD is receiving insufficient critique. And yet this discourse exerts a determining influence on the development of genetics and reproductive health policies, as well as on the international legal and ethical measures now being devised to govern genomics, procreatics, the research on embryonic stem cells, and so on. In this context, our research attempts to highlight the need to consider a segment of the population often forgotten or ignored by policymakers and bioethics experts, the patients who live with severe genetic disorders. Methods: Ethnographic research in the 3 centres accredited for PGD in France: 990 hours of participant observation at PGD clinics and laboratories 79 semi-structured interviews (40 patients and 39 researchers, physicians, and health professionals). Analysis of legal and ethical literature on PGD. Results: The rhetoric employed in current policy debates, which center on concerns about eugenics and discrimination, disregards technical and clinical realities and patients experience. Furthermore, the often invoked notion of the perfect child bears little relation to parents desire to have a child free of the disease they themselves carry. Finally, PGD must be differentiated from in vitro fertilization. Conclusion: The need for an evidence-based discussion is urgent, but the debate should be transparent and include patients and their families. This inclusion could contribute to the development of a realistic and workable normative structure. It is urgently necessary to develop and sustain ethical norms that take into account patients particular context and needs, within a realistic analysis of the risks and benefits for the future child, the parents, and society.

Allele specific expression implicates *MMAB* as the most likely functional gene at the chromosome 12 locus associated with plasma HDL-cholesterol (HDL-C) level. *M. P. Fogarty[1], L. J. Scott[2], R. Xiao[2], K. L. Mohlke[1], FUSION study investigators* 1) Dept Genetics, U. North Carolina, Chapel Hill, NC; 2) Dept of Biostatistics, U. Michigan, Ann Arbor, MI.

Recent genome wide association studies (GWAS) have identified numerous complex trait susceptibility loci; however, the identity of the underlying susceptibility gene(s) and functional variant(s) often remains elusive. Our recent GWAS identified a novel locus on chromosome 12 strongly associated with plasma HDL-C levels ($p=3.4 \times 10^{-8}$; Willer et al, Nat Genet, 40;161). This locus spans at least 4 genes, including 2 strong biological candidates, and contains 54 strongly associated SNPs. We aim to identify the susceptibility gene and ultimately the underlying functional SNP(s). A functional SNP at this locus might play a regulatory role and could act in *cis* to affect gene expression, mRNA processing and stability, or translation. To detect the presence of *cis*-acting regulatory SNPs, we measured allelic expression of five genes in human hepatocytes (n=40 samples). Individuals heterozygous for regulatory variants affecting gene expression or mRNA levels should display allelic expression imbalance (AEI). Using 2-3 transcribed SNPs per gene as markers of AEI, allele-specific expression was measured in the cDNA of heterozygous samples by real-time PCR. We also quantified gene expression in each sample. Significant AEI was observed for 2 SNPs in *MMAB* ($p < 1.1 \times 10^{-8}$). Based on the pattern of linkage disequilibrium, these data suggest that the allele associated with higher HDL-C level is correlated with lower *MMAB* expression. Additionally, preliminary data quantifying gene expression in all samples is consistent with this direction of effect. In contrast, no AEI was observed in 4 other genes tested suggesting that *MMAB* is the most likely susceptibility gene at this locus. These results demonstrate that measuring AEI by real-time PCR has good potential to help identify functional genes in large association regions. We are working to identify the SNP most likely responsible for the AEI in *MMAB* using *in vitro* approaches to test for mRNA stability, transcriptional activity and protein-DNA interactions.

Identifying transcriptional targets of Dlx5 during early development of the mouse inner ear. *SA. Sajan[1], M. Warchol[2], M. Lovett[1]* 1) Division of Human Genetics and Dept of Genetics, Washington Univ School of Medicine; 2) Dept of Otolaryngology.

Development of the endolymphatic duct as well as the anterior and posterior semi-circular canals is compromised in mice lacking the gene *Dlx5*, a member of the Distal-less family of homeobox transcription factors. Its expression begins in the early otic vesicle, and the mutant phenotype becomes visually discernible at around embryonic (E) day 10. Because transcriptional targets of this gene in the developing inner ear are unknown, we sought to identify these targets by expression profiling otic vesicles microdissected from wild-type and *Dlx5*-null mouse embryos at stages E10 and E10.5 on Affymetrix arrays. We identified 293 genes differentially expressed by 1.5-fold or more between the two genotypes in one or both stages. Promoter analysis of the differentially expressed genes revealed 73 with at least one known Dlx5 binding site, of which 18 changed expression only in E10 or in both stages suggesting an earlier interaction with Dlx5. Of these 73 genes, expression patterns of six (*Isl1*, *Apcdd1*, *Pax8*, *Bmp4*, *Bmper*, and *Lrrtm1*) are known to overlap with that of Dlx5 in the developing otic vesicle whereas 19 (including *Otx2*, *Col1a1*, and *Lrrtm1*) have human orthologs whose promoters also contain at least one Dlx5 binding site. We then searched the promoters for any 12bp motifs shared by two or more genes to discover novel Dlx5 binding sites. We found 710 unique motifs of which 33 were also present in promoters of human orthologs, 15 that possessed homeodomain response elements required for binding to homeodomain-containing factors, and another 15 motifs that were found in promoters of genes that also contained known Dlx5 binding sites. Only one gene - Lrrtm1, whose expression is localized in the endolymphatic duct - satisfied all these three criteria, thereby making it a prime downstream candidate of Dlx5 in the otic vesicle. We are currently testing for a direct interaction of Dlx5 with these motifs within the genome by expressing it in an otic vesicle-derived cell line, carrying out chromatin immunoprecipitation using a Dlx5 antibody, and DNA sequencing the immunoprecipitated DNA.

Comprehensive copy number variant screen in hereditary peripheral neuropathies. *J. Huang[1], J. Price[1], G. Montenegro[1], G. Wang[1], X. Wu[2], J. Vance[1], M. Shy[2], S. Züchner[1]* 1) Miami Institute for Human Genomics, University of Miami, Miami, FL; 2) Department of Neurology, School of Medicine, Wayne State University, Detroit, MI.

Hereditary peripheral neuropathies present a group of clinically and genetically heterogeneous entities. All known forms, including the various forms of Charcot-Marie-Tooth disease (CMT) are characterized as Mendelian traits and over 30 genes have been identified thus far. It is well known that copy number variants (CNV) are involved in many diseases, including CMT type 1A. We speculate that hereditary peripheral neuropathy patients without missense mutations might instead carry rare CNVs in known genes, causing the disease phenotype. In this study, we investigated CNVs in 37 genomic regions harboring known genes for hereditary peripheral neuropathies (including the 17p12 duplication region) with NimbleGen 4-plex 385K CGH arrays in a set of 96 patients excluded for missense mutations in the majority of these genes. We identified two genomic regions with frequent CNVs (>20%): 1p35.1 (chr1:33,055,551-33,056,479, 5UTR region of YARS (DI-CMTC), 21%) and 8p23.3 (chr8:1,822,940-1,826,272, intronic region of ARHGEF10, 26%). Though frequent, these CNVs have not been reported previously. Because hereditary peripheral neuropathies are Mendelian diseases, common CNVs are unlikely to be causative for the disease phenotype. However, we also identified 91 regions with rare copy number changes (only gained or lost in a single sample), 18.7% of which covered exonic regions, affecting 11 genes for hereditary peripheral neuropathies. In summary, we performed the first high-density CNV study of hereditary peripheral neuropathy genes in 96 index patients. We have identified genomic regions that are frequently altered in our patient group, as well as rare changes that only occurred in a single patient. The significance of these aberrations is being further investigated.

Runs of Homozygosity Suggest a Role for Rare, High-Risk, Recessive Alleles in Bipolar Disorder. *F. J. McMahon, C. J. M. Steele, N. Akula for the BiGS Consortium* MAP Genetics, NIH/NIMH, Bethesda, MD.

Bipolar disorder is a common mental illness characterized by high heritability and non-mendelian inheritance. Previous studies have suggested the involvement of multiple genes, but so far only common, low-risk alleles have been identified. Empirical and theoretical analyses suggest that rare, high-risk alleles may also play a role. We investigated this hypothesis by searching for extended runs of homozygosity (ROH) among 1030 cases with bipolar I disorder and 1021 healthy controls, all of European origin, who were collected by the NIMH Genetics Initiative and genotyped with the Affymetrix 6.0 SNP array under the auspices of the Genetic Association Information Network (GAIN). Data were cleaned and filtered by a consortium data management committee and obtained by application to dbGAP. Analyses were carried out using Plink 1.01. Results were parsed by use of a PERL script (available upon request). A total of 725,282 markers passed all quality filters. There was no evidence of inbreeding in cases (median Wrights F = -0.0006271) or controls (median Wrights F= -0.0001372). We defined ROH as contiguous regions of homozygosity involving at least 10 adjacent SNPs. A total of 1376 ROH meeting these criteria were detected. ROH were significantly more common in cases than controls ($p < 0.0001$). 62 distinct ROH occurred at least 3 times more often in cases; only one ROH occurred at least 3 times more often in controls. ROH that were more common in cases averaged 102 kb and involved a mean of 28 SNPs. The largest ROH, seen in 6 cases and 1 control, spanned over 200 SNPs in a ~700 kb region on 5p13 that contains the annotated genes FLJ23577, IL7R, and CAPSL, and part of the coding region of UGT3A2, a glycosyltransferase. Other ROHs spanned KCNQ5, TACR1, DPP10, SDCCAG10, ZNF365 and CTNNA3, all of which have been previously implicated in genetic association studies of bipolar disorder. Preliminary analyses suggest that the majority of these ROH are not due to deletion polymorphisms. We conclude that ROH are more common in bipolar disorder than in healthy controls. This finding may reflect a contribution of rare, high-risk recessive alleles to this polygenic disorder.

Profile of Genetic and Metabolic Disorders in Puerto Rican Population. *S. Carlo[1,2], N. Arciniegas[4], A. Quintero[2], N. Ramirez[5], J. Flynn[5], M. Torres[6], A. S. Cornier[1,3]* 1) Dept. of Molecular Medicine, La Concepcion Hospital, San Germán, PR; 2) Dept. Biochem, Ponce Sch Med, Ponce, PR; 3) Dept. of Internal Medicine, Univ. of PR School of Medicine, Rio Piedras, PR; 4) Dept. of Pediatrics, La Concepcion Hospital, San Germán, PR; 5) Dept. of Orthopaedic, La Concepcion Hospital, San Germán, PR; 6) Epidemiology Center, Puerto Rico Dept. of Health, San Juan, PR.

Puerto Rico is an island located in the Caribbean with a population of approximately 4 million habitants.High incidence rates for disorders such as oculocutaneous albinism, Hermansky-Pudlack syndrome, Spondylothoracic Dysostosis (Jarcho-Levin syndrome) and Bardet-Biedl syndrome. However, there is no data regarding prevalence of genetic and metabolic disorders in Puerto Rico except, PKU, galactosemia, congenital hypothyroidism, sickle cell, congetial adrenal hyperplasia that are included in the newborn screening program. We reviewed 5,750 medical records of genetic clinics at the Pediatric Centers of Puerto Rico. The data was tabulated and analyzed in order to perform a statistical analysis so the prevalence and epidemiological profile of genetic and metabolic patients in Puerto Rico could be established. The groups were analyzed independently using the previous assigned number for the diagnoses, age, sex, reason for referral, municipality and management. Primary diagnoses were organized in general categories revealing the following results. Metabolic Disorders with 22.7%, Genetic disorders 29.9%; Congenital malformations 11.1%; Developmental Disease 18.3%; Behavioral Disorder 11.5%; and others 76 6.4%.The frequency of 45 specific diagnosis was also calculated and determined for different regions of Puerto Rico.The results of this research may prove to be useful for the determination of health care public policies, additions to the newborn screening program, assignment of resources in Puerto Rico. These data will serve as guide to health care services in states with a large amount of Puerto Rican population (NY, NJ, IL, CT, FL and TX) in the mainland US and may prove useful as a reference to compare with other Hispanics population in the mainland United States.

Molecular and Developmental Insights into Craniofacial Abnormalities in Tricho-Rhino-Phalangeal Syndrome (TRPS). *D. Napierala[1], K. Choudhry[2], E. Munivez[1], B. Lee[1,3]* 1) Dept Molecular & Human Genetics, Baylor College of Medicine, Houston, TX; 2) Dept Pediatric Endocrinology, Baylor College of Medicine, Houston, TX; 3) Howard Hughes Medical Institute.

Tricho-rhino-phalangeal syndrome (TRPS) is a dominantly inherited craniofacial and skeletal dysplasia caused by mutations involving the *TRPS1* gene. TRPS patients present with sparse hair and skeletal abnormalities reflecting disturbed endochondral bone formation. Moreover, TRPS patients have distinctive craniofacial features, some possibly attributable to defects of soft tissue, but the bulbous nose and micrognathia point to skeletal abnormalities. The *TRPS1* gene encodes for a zinc-finger transcriptial repressor TRPS1 that belongs to the GATA family of transcription factors. To understand the molecular mechanism of the TRPS craniofacial abnormalities we study *Trps1* mutant mice with an in frame deletion of the DNA binding domain of Trps1 (*Trps1GT/+* mice). Heterozygous mutations of the *Trps1* gene in mice phenocopy characteristic TRPS features. The heterozygous *Trps1GT/+* mice develop postnatally a mild craniofacial phenotype, while the homozygous *Trps1GT/GT* mice present with more severe craniofacial abnormalities including the cleft palate. Analyses of Trps1 expression during craniofacial development demonstrated that Trps1 is specifically expressed in perichondrium of all primordia of endochondral bones, cartilage of nasal capsule, tracheal cartilage and palatal shelves of maxilla. Moreover, high Trps1 expression was observed in primordia of all types of hair follicles, dental mesenchyme, developing ear and eye lids. Our analyses of the Trps1 function in cartilage demonstrated that Trps1 regulates chondrocyte proliferation and development. Moreover, Trps1 physically interacts with Runx2 and represses Runx2 during endochondral bone formation. Interestingly, we observed increased Indian hedgehog (Ihh) expression and signaling in Trps1 mutant mice. Therefore, Trps1 may regulate craniofacial development through interaction with hedgehog signaling pathway.

Animal models for functional evaluation of genes in autism spectrum disorders. *J. Buxbaum, N. Dorr, G. Elder, A. McInnes, M. Gama Sosa, T. Sakurai* Psychiatry, Mount Sinai Sch Medicine, New York, NY.

Autism is a genetic disorder of complex etiology. Autism can result from inherited and de novo chromosomal abnormalities and single gene mutations. These forms of autism, associated as they are with a single locus, are very amenable to modeling in mice. Our laboratory has developed a moderately high throughput behavioral screen to search for deficits in mice that might relate to key aspects of the autism phenotype. The screen includes an observational component, evaluation of spontaneous and elicited behaviors, and evaluation of complex behaviors including social interactions, learning, and memory. In parallel, we have generated 8 distinct murine models that have relevance to autism. The models target genes likely involved in social communication, social behavior, synaptic function, etc., and include knockouts and/or transgenics of AGC1, FOXP2, OXTR, AVPR1A, and GTF genes. We have applied our behavioral assays to these animal models, while also carrying out neuropathological analyses as well. In multiple models we see evidence for abnormalities in Purkinje cells. We also see deficits in social communication and social memory in two distinct models. We will describe the specific steps in the behavioral screen using results from these autism models.

Genetic testing of GAA: a prompt and reliable laboratory diagnosis of Pompe disease. *B. Tinkle[1], S. Peters[2], K. Zhang[1], N. Leslie[1]* 1) Div Human Genetics, Cincinnati Children's Hosp, Cincinnati, OH; 2) University of Cincinnati School of Medicine, Cincinnati, OH.

Pompe disease is due to the deficiency of acid -glucosidase resulting in glycogen accumulation in lysosomes. Diagnosis is suspected clinically; hypertrophic cardiomyopathy in the infantile form or muscluar weakness in the late-onset form. In affected infants, early recognition and initiation of ERT is paramount. Confirmation of Pompe can be done by histological demonstration of glycogen storage, enzyme deficiency, and/or GAA sequence analysis. Although enzyme testing is the gold standard, some difficulties exist. The enzyme is heat labile and can be artificially low on dried blood spots. WBCs contain maltase glucoamylase which can cause false negative results. The more sensitive and specific assay is enzyme analysis on fibroblasts but this can take several weeks. However, DNA analysis is very sensitive and can be done relatively quickly (McCready et al., 2007). DNA analysis has the additional benefit of being used predictively in prenatal testing as well as carrier testing. We developed a comprehensive DNA sequence analysis complemented by PCR fragment analysis of the common exon 18 deletion. 65 samples with features of Pompe and either an enzymatic or histological diagnosis were analyzed. Of the 65, 129 alleles were identified (99.2% sensitivity). Combining the data from McReady et al., (2007), 170 pathogenic alleles were identified among 172 alleles with an overall sensitivity of 98.8%. In addition, two patients were genotyped as normal that had reduced enzyme from dried blood spots. Dried blood spots and DNA sequencing offer quick and convenient confirmatory testing of Pompe disease. Dried blood spots may be heat and humidity sensitive and a control enzyme should be measured along with the GAA activity (The Pompe Disease Diagnostic Working Group, 2008). Further, enzyme activity in the late-onset form can be near-normal or normal in any of the enzyme assays. As neither test is infallible, both tests have advantages and are very sensitive for the infantile form but both likely have more, albeit minor, limitations in confirming the late-onset form.

Inter-individual variation in retrotransposition-competent human LINE-1 content. *C. R. Beck[1], P. Collier[2], C. Macfarlane[2], M. Malig[3], J. M. Kidd[3], E. E. Eichler[3], R. M. Badge[2], J. V. Moran[1]* 1) Departments of Human Genetics and Internal Medicine, University of Michigan Medical School, Ann Arbor MI; 2) Department of Genetics, University of Leicester, Leicester, UK; 3) Department of Genome Sciences and Howard Hughes Medical Institute, University of Washington, Seattle, WA.

L1 retrotransposition events continue to impact human DNA. The average human genome contains ~80-100 full-length, retrotransposition-competent L1s (RC-L1s); however, relatively few highly active or hot L1s appear to account for the bulk of human L1 retrotransposition activity. The currently active family of human L1s, the Ta-subfamily, arose ~4 MYA. These elements contain diagnostic sequence variants that allow their discrimination from older L1s that have accumulated during human evolution. Many hot L1s are present at low allele frequencies, suggesting that they may be under-represented in the human genome reference (HGR) sequence. Here, we combined genomic, genetic, and molecular biology approaches to uncover novel RC-L1s. First, we employed a fosmid-based end sequencing approach to identify insertion polymorphisms in the genomes of six individuals of unknown, Chinese, Japanese, European, and Yoruban (2 individuals) descent. Next, we used a PCR/Southern blot strategy to screen candidate fosmids for full-length, Ta-subfamily L1s, and designed genotyping assays to determine their allele frequencies. Finally, we used a cultured cell assay to determine if the candidate L1s were active in vitro. We identified 62 full-length L1s that are absent from a database of known L1 polymorphisms, and have assayed 46 of these for retrotransposition. Remarkably, 26 elements (~57%) were highly active, hot L1s. Preliminary analyses indicate that 5 of these hot L1s are absent from a CEPH genotyping panel consisting of 129 unrelated individuals, and may represent recent and/or population restricted RC-L1s. Thus, we have developed a powerful system to identify hot L1s from individual human genomes of diverse geographic origin. The data suggest that hot L1s are under-represented in the HGR, are far more prevalent than previously appreciated, and that their mobility continues to contribute to inter-individual genetic variation.

Mapping genetic susceptibility loci of hypocholesterolemic autism using nonparametric multipoint linkage analyses. *E. Tierney[1], Y. Kim[2], K. Weissbecker-Remer[3], F. D. Porter[4], J. E. Bailey-Wilson[2]* 1) Kennedy Krieger Institute, Baltimore, MD; 2) National Human Genome Research Institute, NIH, Baltimore, MD; 3) Tulane University, New Orleans, LA; 4) National Institute of Child Health and Human Development, NIH, Bethesda, MD.

Different loci have been identified as potential susceptibility genes for autism spectrum disorder (ASD) in previous genome-wide screens of multiplex families. To search for genes related to ASD, linkage analyses and association studies have been performed. Based on the previous finding that some individuals who had 1 or more family members with ASD were found to have low cholesterol levels, we hypothesized that either autism or other ASD subjects with hypocholesterolemia may have different genetic susceptibility loci. We obtained microsatellite genotype data for 390 loci on families with at least 2 children with ASD and hypocholesterolemia who donated blood to the Autism Genetic Resource Exchange. To analyze hypocholesterolemic probands, we defined 2 family groups: 1) cholesterol levels greater than 2 SD (standard deviation) below the mean (47 families; below-2SD), and 2) less than 100 mg/dl (28 families; below-100). In each group we analyzed different subsets depending on disease diagnosis: 1) both sibs had autism or 2) one or more sib had Not Quite Autism or Broad Spectrum using nonparametric multipoint linkage method. Additional analyses were performed on groups of male-only sibs and female-containing sibships in each subset. In the below-2SD autism families, D10S1412 at 10p14 shows nominal significance (P value of NPL score 0.005, Kong & Cox LOD=2.02); this is near a site for DiGeorge syndrome/velocardiofacial syndrome, a disorder associated with ASD, and a site of terminal deletion associated with ASD. Female containing sib pairs in below-100 autism families had nominally significant linkage at markers D5S822 (p value= 0.005, Kong & Cox LOD=1.37) and D5S1969 (p value= 0.002, Kong & Cox LOD=1.5) at 5q11.1-11.2. We cannot conclude that these loci have suggestive linkage evidence for sib pair analysis. However, they suggest that further analyses with more samples of hypocholesterolemic ASD families are needed.

Genetic variants on INSIG2 and PPARG and their associations with obesity related traits in an isolated population from Croatia. *G. Sun[1], H. Cheng[1], H. Xi[1], S. R. Indugula[1], P. Pal[1], J. Mallik[1], G. Zhang[1], R. Chakraborty[1], P. Rudan[2], R. Deka[1]* 1) Dept Environmental Health, Univ Cincinnati, Cincinnati, OH; 2) Institute for Anthropological Research, Zagreb, Croatia.

In an ongoing study on genetics of Metabolic Syndrome (MS) in an isolated population, we tested for association of tagging SNPs in two genes, INSIG2 and PPARG, with 28 obesity related anthropometric and biochemical traits. The study population, from an island of the eastern Adriatic coast of Croatia, has a high prevalence of MS. Since their migration to their current locale from the mainland Croatia in the 15th century, this population of Slavic origin has remained relatively isolated. Sequence variants in INSIG2 and PPARG are associated with obesity and type 2 diabetes. Forty one tagging SNPs (12 on INSIG2 and 29 on PPARG) were chosen (r2 >0.8, MAF 0.05) using the Caucasian HapMap database. Genotyping was performed on DNA from 870 subjects of age >18 years. We used ANOVA to test for differences between genotype groups. There was no significant association of PPARG variants with any of the traits. However, three variants on INSIG2 (rs1352083, rs13393332, rs2042492) were significantly associated (p = 0.0004-0.0005) with the tricep skinfold measures. Although we did not find association with other anthropometric measurements and biochemical traits, these results indicate that genetic variation on INSIG2 influences obesity related phenotypes. Supported by grant R01DK069845.

Improved Microarray-Based Genomic Selection (MGS) of Targeted Human Diploid and Haploid X-Chromosome Sequences for Next Generation Sequencing. *D. Okou[1], K. Meltz-Steinberg[1,2], A. Locke[1,3], P. Atri[1], V. Patel[1], M. Zwick[1,2,3]* 1) Dept Human Genetics, Emory Univ Sch Medicine, Atlanta, GA; 2) Graduate Program in Population Biology, Ecology and Evolution, Emory Univ, Atlanta, GA; 3) Graduate Program in Genetics and Molecular Biology, Emory Univ, Atlanta, GA.

Microarray-based Genomic Selection (MGS) can enrich targeted genomic regions for subsequent resequencing. Here we address two significant questions regarding the robustness of MGS: 1) the ability to accurately identify genotypes in diploid genomic regions and 2) the extent of coverage for large targeted regions. The Illumina Genome Analyzer (IGA) was used as platform for resequencing. To test the former, MGS arrays were used to enrich 300kb of coding and noncoding sequences from 1.7MB region on the human X chromosome in 10 female HapMap samples. Our improved protocol yielded ~2500-fold with 80X average sequence coverage in a single lane of IGA sequencing. In order to compare our MGS/IGA calls with HapMap calls, we focused our attention on ~344 SNPs genotyped in the HapMap (3190 genotypes total). Our average genotype-calling rate at all sites (97.8%) compared well with HapMap (98.7%). At homozygous genotypes, MGS/IGA called 98.4% of sites (HapMap 98.8%), whereas at heterozygous genotypes we called 84.2% of sites (96.6%). Our call rate at heterozygous sites significantly exceeds that previously reported (30%). When both technologies make a call at a given variable site, assuming that the HapMap data contains no errors, we observe an overall agreement of 96.3%. At homozygous sites, the MGS/IGA accuracy (96.7%) is nearly equivalent to that of HapMap (99.7%), while at heterozygous sites, the MGS/IGA accuracy is lower (84.8%) than that of HapMap (98.7%). To test the latter, an initial custom-designed MGS arrays (385k oligos) were used to enrich for 7066 exons (~2.4MB) on the human X-chromosome in 10 male HapMap samples. Data from a single lane of IGA sequencing yielded ~180-fold enrichment with 7.5 X average coverage in ten experiments. Our data suggest increased probe density will be required to obtain high fold enrichment and sequence coverage for large regions.

Identification of Diabetic Retinopathy Genes through a Genome-Wide Association Study among Mexican-Americans from Starr County, Texas. *Y. P. Fu[1], D. M. Hallman[1], V. H. Gonzalez[2], B. E. K. Klein[3], R. Klein[3], M. G. Hayes[4], N. J. Cox[4,5], G. I. Bell[4,5], C. L. Hanis[1]* 1) Human Genetics Center, School of Public Health, The University of Texas Health Science Center at Houston, Houston, Texas; 2) Valley Retina Institute, McAllen, Texas; 3) Department of Ophthalmology and Visual Sciences, Madison Medical School, The University of Wisconsin, Madison, Wisconsin; 4) Department of Medicine, University of Chicago, Chicago, Illinois; 5) Department of Human Genetics, University of Chicago, Chicago, Illinois.

To understand genetic susceptibility for diabetic retinopathy, a genome wide association study was conducted using the Affymetrix GeneChip Human Mapping 100K Set in a sample of 284 Mexican-Americans with type 2 diabetes from Starr County, Texas. All subjects had detailed ophthalmologic examinations including stereoscopic fundus photography and standardized retinopathy grading. A series of single and multi-marker association tests were performed to identify underlying genetic susceptibility for diabetic retinopathy. In initial single marker analysis, we found 10 loci associated with diabetic retinopathy with p-values less than 10^-4. Interestingly, 3 of these top 10 signals all mapped to STAC (src homology 3 and cysteine rich domain) located on chromosome 3. Furthermore, sliding-window haplotype analyses showed several regions with consistent significance in windows ranging from 2 to 8 SNPs. In particular, haplotypes containing SNPs residing in the intron region of TECTB (tectorin beta) on chromosome 7 reached statistical significance after Bonferroni correction. None of the SNPs with suggestive evidence in our single marker and haplotype analyses were in traditional candidate genes for diabetes or its complications. We are now conducting imputation analysis to expand coverage across the genome, and are turning to various multi-marker/gene/pathway analytical approaches to more fully extract the genetic risk and underlying biology of diabetic retinopathy.

Array-CGH analysis in a cohort of 112 Italian patients with MCA/MR. *E. Katzaki, FT. Papa, MA. Mencarelli, K. Sampieri, V. Uliana, M. Pollazzon, A. Marozza, I. Longo, F. Ariani, I. Meloni, F. Mari, A. Renieri* Medical Genetics, University of Siena, Siena, SI, Italy.

We have investigated 112 patients with mild to severe mental retardation associated to facial dysmorphisms and/or congenital anomalies. All patients but one (47,XXX) had a normal karyotype and have been evaluated by clinical geneticists (AR and FM) who excluded a recognizable syndrome on a clinical ground. Using either 44K or 105K oligo Array-CGH private imbalances were detected in 35 out of 112 patients. In 17 cases (15%) a private rearrangement was inherited from one healthy parent (Mencarelli et al Eur J Med Genet in press). In 18 cases (16%) the rearrangement was de novo: 5 were novel deletions, 2 were complex dup/del rearrangements and 11 were known syndromes in atypical cases. The last group included three cases of 22q11 deletions, the shortest 4p- and 6q- known in the literature, and two cases of Potocki-Lupski. Two complex rearrangements were found in two peculiar patients. One patient with bifid thumb, hypertonia, tricuspid atresia, aggressive behavior and normal karyotype, had a 9 Mb interstitial deletion on 3q including TP63 and a 4Mb duplication of chr 21. The other patient with microcephaly, iris coloboma and hypoplastic toes had a 4.5 Mb terminal 9pdel and a 3 Mb terminal 17pdup, reciprocal of Miller-Dieker, due to an unbalance of a balanced translocation present in the mother. The 5 novel de novo deletions ranged between 2.6 and 13.9Mb and they overlapped with polymorphic regions for an extent of 5-75% (Pescucci et al Eur J Med Genet 2007, Mencarelli et al Am J Med Genet 2007 and Papa et al. Am J Med Genet, 2008). Only two (6q24.3-q25.1 and 7q36.1-q36.2) are flanked by LCRs (Caselli et al, Eur J Med Genet, 2007 and Caselli et al Am J Med Genet, 2008). An accurate search of the literature allowed to identify patients with overlapping deletions. Comparative analysis of the phenotype of these patients with our patients suggested that a specific phenotype of these syndromes may be defined. These characteristics should be taken into account in order to identify additional patients.

***MTHFR* polymorphisms and genome-wide DNA methylation.** *CW. Hanna, MS. Peñaherrera, MS. Kobor, WP. Robinson* Department of Medical Genetics, University of British Columbia, Vancouver, BC, Canada.

The folate pathway provides a link between the environment and epigenetics, with dietary folic acid being metabolized into methionine that can then be used by methyltransferases to methylate DNA. A low folate diet and folate pathway polymorphisms can result in elevated homocysteine and decreased methionine levels. Both have been previously reported to be associated with altered DNA methylation. In addition, genetic variants in the folate pathway have been linked to hypertension, preeclampsia, Down syndrome, birth defects, and recurrent miscarriage in some populations. These effects could be due to hyperhomocysteinemia itself or the potential effects on DNA methylation. To identify DNA methylation changes associated with functional polymorphisms in the folate pathway we extracted DNA from whole blood from females of reproductive age. Genotypes for the -C677T and -A1298C polymorphisms in methylenetetrahydrofolate reductase (*MTHFR*) were determined using pyrosequencing on the Biotage PyroMark MD^{TM}. Global methylation was measured using pyrosequencing of a consensus LINE-1 element sequence (N=84) and by the Illumina Goldengate^{TM} Methylation Bead Array (N=35), which assesses the methylation level at 1505 CpG sites at over 800 genes. Global methylation did not differ by *MTHFR* genotype based on LINE-1 methylation or average methylation measured by Illumina Array. Nonetheless, some regions of the genome may be more susceptible to altered methylation in individuals homozygous for polymorphisms encoding a thermolabile MTHFR. Seven CpG sites were significantly associated with the *MTHFR* -677TT genotype ($p<0.01$), while twenty CpG sites were significantly associated with the *MTHFR* -1298CC genotype ($p<0.01$). Several interesting candidate genes are being followed up further by a targeted approach in a larger sample population. While we did not observe global alterations in methylation associated with susceptible genotypes, variation in dietary folate intake may have prevented genotype-associated changes from being apparent in our sample population.

Method for quality control and validation of multiplex ligation-dependant probe amplification (MLPA) assays.

G. Pont-Kingdon[1], L. S. Chou[1], C. Vaughn[1], T. Lewis[1], F. Gedge[1], E. Lyon[1,2] 1) ARUP Institute for Clinical and Experimental Pathology, ARUP Laboratories, Salt Lake City, UT; 2) Pathology Department , University of Utah, Salt lake City, UT.

Deletions and duplications of single or multiple exons in specific genes are associated with human diseases. These types of mutations are detected in multiple laboratories by MLPA, which has the advantages of a resolution at the exonic level and a high multiplexing capability. MLPA uses a series of exon-specific probes that hybridize to their template prior to PCR amplification. Kits containing a mixture of exon-specific probes targeted to the gene of interest and control probes that hybridize to other genomic areas are commercially available (MCR-Holland, Amsterdam). Only validation results from positive samples can truly confirm that the probes listed in the kits correspond to the targeted exons, as negative (WT) samples contain all the genome and hybridize equally to any human DNA probes. Characterized positive samples can be extremely rare and difficult to obtain for validation purposes. Additionally, deletion of multiple exons in a sample will not interrogate each probe individually. We have designed an approach using master mixes of exon-specific PCR products to unequivocally verify each probe. As an example of the method, data will be presented using MLPA testing for a series of genes for which positive samples were difficult to obtain: COL4A5 (Alport syndrome), MSH6 and PMS2 (Lynch syndrome), FVIII (hemophilia A) or as a method for QA/QC of MLPA for CFTR (cystic fibrosis) and ENG and ACVRL1 (hereditary hemorrhagic telangiectasia). Data indicates that this easy and rapid approach can be used for analytical validation of the probes and for routine QA/QC of MLPA kits in absence of positive samples.

Fast and Robust Association Tests for Untyped SNPs in Case-Control Studies. *M. P. Epstein[1], A. S. Allen[2], S. Griffiths[3], F. Dudbridge[3], G. A. Satten[4]* 1) Dept Human Genetics, Emory Univ, Atlanta, GA; 2) Department of Biostatistics and Bioinformatics, Duke University, Durham, NC; 3) MRC Biostatistics Unit, Cambridge, UK; 4) Centers for Disease Control and Prevention, Atlanta, GA.

Genomewide association studies of complex diseases typically genotype and analyze a set of tagSNPs that effectively capture genetic variation across the genome. Nevertheless, many such studies have substantial interest in testing SNPs that are not genotyped formally in the test sample. Such analyses of untyped SNPs can assist in signal localization and permit cross-platform comparison of results from different studies. While such untyped analyses might initially appear intractable, a study can extrapolate information on an untyped SNP in a sample using the observed tagSNP data coupled with external haplotype-based information on all SNPs (both typed and untyped) from an appropriate reference catalogue of human genetic variation (such as one of the samples from the International HapMap Project). Using this logic, we propose a novel statistical approach for testing untyped SNPs in case-control genomewide association studies. We base our approach on an efficient-score function derived from a prospective likelihood of data, which facilitates easy modeling and testing of untyped SNPs and covariates. In addition, we show both theoretically and empirically that our approach is robust to an inappropriate choice of reference sample for inference of untyped SNPs and, further, does not require adjusting for the additional variability in estimating haplotypes from genotype data. As a result, our efficient-score test is computationally much faster than existing approaches for untyped analysis and has a closed form even for stratified data that allows for easy programming in existing software packages. Regarding this former strength of our method, we can analyze ~1.6 million untyped SNPs in a case-control dataset of 1000 subjects in ~90 minutes on a single Windows processor. At the same time, we show using simulated data that our approach has near-equivalent performance compared to the popular hidden-Markov methods of untyped analysis.

Higher than Expected Frequency of a Third Mutant Allele in a Multiethnic Population Affected with Bardet-Biedl Sydrome. *G. Billingsley[1], J. Bin[1], E. Héon[1, 2]* 1) Dept Genetics & Genome Biol, Hosp Sick Children, Toronto, ON, Canada; 2) Dept of Ophthalmology and Vision Sciences, The Hospital for Sick Children, University of Toronto.

Bardet-Biedl syndrome (BBS: OMIM 209900) is an autosomal recessive, genetically heterogeneous disorder characterized by the primary features of progressive retinal dystrophy, obesity, polydactyly, renal malformations, cognitive impairment and genital abnormalities. Its incidence is approximately 1 in 150,000 individuals in European populations, while the carrier frequency is estimated to be 1 in 50 (Laurier et al. Eur J Hum Genet (2006) 14, 1195). To date 12 BBS genes have been identified. Mutational analysis of the 12 BBS genes was performed on a BBS patient cohort (77 cases and 62 index cases) of mixed ethnicity. Family segregation and control screening (n=150) confirmed the pathogenicity of novel sequence changes. Together, the 12 BBS genes accounted for 68.5% of putative pathogenic changes in our index cases. BBS1 and BBS10 were major contributors to BBS in our patient cohort, each accounting for 20% of the mutational load. BBS6 and BBS12 each accounted for 6.5% of the total mutational load. In individuals with two bona fide mutations in a single BBS gene, we observed a much higher frequency of a third mutant allele than the predicted 2% carrier rate (23.3% of all cases and 19.3% of index cases). However, this observed frequency may actually be an underestimate of the carrier frequency in our cohort, since not all genes have been analyzed in all patients. In conclusion, our results show that BBS1 and BBS10 play a major role in our patient cohort. The observed enrichment of carriers of a third mutant allele supports the genetic complexity of BBS. It is suspected this third allele may have an effect on the clinical manifestation of the disease.

Up to 48-Months on Treatment: Open-Label Phase I/II Long-Term Study of Enzyme Replacement Therapy (ERT) with velaglucerase alfa in Patients with Type 1 Gaucher Disease. *A. Zimran[1], G. Altarescu[1], M. Phillips[1], K. Bhirangi[2], R. Mensah[2], D. Elstein[1]* 1) Gaucher Clinic, Shaare Zedek Medical Center, Jerusalem, Israel; 2) Shire Human Genetic Therapies, Inc., Cambridge, MA, USA.

AIM: To evaluate the long-term safety and clinical activity of velaglucerase alfa, Gene-Activated human glucocerebrosidase, an investigational ERT for patients with type 1 Gaucher disease. BACKGROUND: velaglucerase alfa is produced in a human cell line and has an identical amino acid sequence to the naturally occurring human enzyme. METHODS: Ten of 11 patients who completed the Phase I/II study enrolled in the long-term extension study. One patient since discontinued treatment for reasons unrelated to velaglucerase alfa. At or after Month 12, all patients qualified (based on meeting at least 2 out of the 4 Therapeutic Goals for ERT in Type 1 Gaucher disease) to begin a step-wise dose reduction from 60U/kg to 45U/kg (13 weeks) and then to 30U/kg. Additionally, home therapy was made available to patients in the extension study. RESULTS: Up to Month 42 on treatment: Most AEs were mild to moderate in severity and include arthralgia, back pain, headache, abdominal pain upper, and pharyngolaynal pain. Notably, no patient has developed antibodies to velaglucerase alfa. Statistically significant mean and mean percent increases in hemoglobin concentration from baseline to Month 42 (2.18g/dL and 19.0%, respectively; p=0.004) and in platelet count (82.1 x 103/mm3 and 149.8%, respectively; p=0.004) were observed. Additionally, statistically significant mean percent decreases from baseline for spleen and liver volume were observed after 33 months of treatment, which indicated further improvement in organ volumes with long-term treatment of velaglucerase alfa (72.3% and 29.6%, p=0.008 and p=0.004, respectively). At Month 30, marked decreases were observed in the mean percent change from baseline in the biomarkers chitotriosidase (83.5%) and CCL18 (57.2%). Up to 48 month data will be presented at the meeting. CONCLUSION: velaglucerase alfa has continued to be generally well tolerated and demonstrated clinical activity in disease parameters in these adult patients with type 1 Gaucher disease.

A case of diphallia and anal atresia. *J. Samanich[1], C. Long[2], S. Nemerofsky[2]* 1) Center for Congenital Disorders, Children's Hospital at Montefiore, Bronx, NY; 2) Section of Neonatology, Children's Hospital at Montefiore, Bronx, NY.

Diphallia, or duplication of the phallus, is an extremely rare disorder, classified into categories of true diphallia (two corpora cavernosa) and bifid phallus (single corpora cavernosa). Several other anomalies have been reported in conjunction with diphallia, including imperforate anus, cryptorchidism, perineal mass, renal anomalies, rectal anomalies, duplicated bowel and duplicated bladder. Case Report: ML was a full term infant delivered after an uneventful pregnancy to a 22-year-old G3P2002 African-American mother and 37-year-old Jamaican father. There was no known family history of birth defects. At delivery, anomalous genitalia and an imperforate anus were noted. Physical examination revealed length 50.5 cm (50%), weight 3550 g (50%) and HC 32.5 cm (5-10%). He had a split metopic suture, a slightly depressed nasal bridge and a left preauricular tag, but generally non-dysmorphic facies. There was a duplicated phallus with two scrotums. The ventral scrotum contained 2 testes and the dorsal scrotum was empty. There was a well-defined gluteal crease, imperforate anus and a 2-vessel cord. Cystourethrogram revealed that the ventral urethra drained to the bladder and the dorsal urethra was atretic. Also noted was a duplicated rectum with the more anterior rectum connected to the bladder by a rectovesical fistula. The bladder was normal. Ultrasound showed normal kidneys, adrenals, spleen and sacrum. A large left hepatic lobe was noted, but otherwise normal liver. MRI of the brain was unremarkable. Echocardiogram revealed significant right ventricular hypertrophy of unclear etiology, with no structural defects. During surgery, he was found to have a high anorectal malformation and sigmoid colostomy was performed. Chromosomal analysis showed 46, XY. MRI of the pelvis to determine if he has true diphallia or a bifid phallus is scheduled. This is a unique case of a duplicated phallus, duplicated rectum, and an imperforate anus without clear genetic or environmental etiology.

Caring for families at risk for sudden death (SD): The organization and early outcomes of a multidisciplinary (M/D) cardiogenetics clinic. *K. O. Pope, E. D. Paljevic, C. A. Walsh, T. McDonald, R. W. Marion* The Montefiore-Einstein Center for CardioGenetics, Bronx, NY.

Background: Family members of SD victims are left with questions and lingering fear that others could have a similar fate. Ten percent of SIDS (Sudden Infant Death Syndrome) and 33% of SUDS (Sudden Unexplained Death Syndrome) result from mutations in cardiac ion channel genes, and the genetic contribution to SD is greater when familial hypertrophic cardiomyopathy (FHC) is also considered. Newly available genetic testing for these conditions makes collaboration between geneticists and cardiologists essential to address all aspects of care. We describe a M/D clinic, the Einstein-Montefiore Center for CardioGenetics. **Patients**: NY metro area families are referred by self, physician, or through a unique partnership with the NYC Office of the Chief Medical Examiner. **Methods**: The clinic includes a nurse practitioner (the clinic coordinator), geneticist & genetic counselor, pediatric & adult cardiologist, and social worker with grief management expertise, and occurs monthly. Cardiac screening and diagnostic tests are performed. The social worker meets each family, assesses needs, and provides support. Clinical geneticists recommend and facilitate appropriate genetic testing. For previously undescribed mutations, *in vitro* phenotyping of the resulting protein is performed to assess pathogenicity. **Results**: During the clinics first 6 months, 15 families have been seen with the following conditions: SIDS, SUDS, near miss SUDS, prolonged QTc, Andersen-Tawil syndrome, arrhythmogenic right ventricular dysplasia, and FHC. Interviews post-visit indicate overall patient satisfaction. **Discussion**: Pitfalls encountered to date include difficulty sending genetic testing on the proband and the identification in one family of a potentially deleterious variant in *SCN5A*. Counseling for these families is complex and challenging. **Conclusion**: Caring for families who have experienced the impact of sudden death requires collaboration among multiple professionals. We present a schema of a M/D cardiogenetics clinic, and report our initial experience.

Genome wide association signals of rare SNP variants are significantly associated with linkage evidence in rheumatoid arthritis. *X. Ke[1], S. Eyre[1], R. Lawrence[2], J. Bowes[1], A. Barton[1], W. Thomson[1], . WTCCC[3], J. Worthington[1], E. Zeggini[2]* 1) ARC Epidemiology Unit, Univ Manchester, UK; 2) The Wellcome Trust Centre for Human Genetics, Univ Oxford, UK; 3) The Wellcome Trust Case Control Consortium, UK.

Multiple genome-wide linkage scans have been carried out for rheumatoid arthritis (RA). Linkage peaks are likely to be caused by rare variants with high penetrance. Using data from the WTCCC study, the largest genome-wide association scan for RA to date, we investigated the overlap between linkage and rare variant association signals across the genome. We included 11 regions with replicating evidence for linkage ($p<0.05$) in independent studies. These linkage regions were delineated by the most significantly associated microsatellites plus 10Mb on each side. The WTCCC Affy500k scan contained 40,482 SNPs with minor allele frequency <5% in 1,860 RA cases and 2,938 controls. We defined 22,344 gene regions across the autosomal genome as intervals 50kb either side of the transcriptional start and end sites. 18,246 of these contained at least one rare variant. Genotypes of all rare variants within a gene were collapsed into a single locus and a global p value was calculated per gene region. We examined the extent of overlap between linkage evidence and rare variant association signals using logistic regression. There was significant association between the -log p values of rare variants in genes and linkage evidence in the genome ($p<0.0001$). The fucokinase gene (FUK), with 4 rare variants, is one example. It is located under a linkage peak on chromosome 16q22.1. A total of 29 of the 1,860 RA cases and 7 of the 2,938 controls carried the rare variant alleles (p value of Fishers exact test = 2.6x10-7). The enzyme involves in the utilization of free L-fucose, which may be important in mediating a number of cell-cell interactions such as blood group antigen recognition, inflammation and metastatis. Therefore, FUK may be an interesting candidate for RA susceptibility. This is the first study to examine the overlap between genome-wide rare variant association signals and replicating linkage peaks for a complex common disease.

Transglutaminase-1 gene mutations in Autosomal Recessive Congenital Ichthyosis: An Update and a Report of 24 Novel Mutations. *M. Herman[1], S. Farasat[1], M. H. Wei[1, 2], O. Toure[1], S. Bale[3], J. Toro[1]* 1) Genetic Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Rockville, MD 20892; 2) Basic Research Program, SAIC-Frederick Inc., Frederick, MD 21702, USA; 3) GeneDx, Inc, Gaithersburg, MD 20877, USA.

Autosomal recessive congenital ichthyosis (ARCI) is a cornification disorder. Mutations in the transglutaminase-1 (TGM1) gene cause ARCI in 55% of affected patients. We characterized the TGM1 mutations reported to date including 24 novel mutations in 26 patients (48 mutated alleles). Of the twenty-four novel mutations 16 were missense (T21A, V209F, R225P, E285K, F293V, I304F, R323W, W342R, V359M, L366P, H405N, M421V, F435V, A560G, R689C, R689H), two frameshift (c.802delG, c.1223_27delACACA), two nonsense (R54X, Q124X), and four putative splice site mutations (c.1-1G>C, c.984+1G>A, c.1159+1G>T, c.2226-2A>G). Missense mutations were the most common and 69% (11/16) occurred within the catalytic core domain. Of the twenty coding novel mutations, five mutations (25%) affected four different arginine codons. Including this study, a total of 117 different mutations have been reported in 282 individuals. Seventy-three (62%) of the observed mutations were missense, twenty (17%) nonsense, nine (8%) deletion, eight (7%) splice site and seven (6%) insertion. Thirty-seven of the mutations (32%) are expected to lead to truncated proteins. All translated exons (2-15) displayed at least one mutation. Exon three was the most mutated, accounting for 14% (16/117) of all mutations. Of the 817 amino acids in TGase-1, 54 (6.6%) are arginines of which (91%) of their codons begin with a CpG dinucleotide. Twenty-nine percent (34/117) of all mutations and 38% (28/73) of missense mutations occurred in arginine residues in TGase-1. Forty-eight percent (35/73) of missense mutations were within CpG dinucleotides and 74% (26/35) of these mutations were C-> T or G->A transitions. The high frequency of mutated arginine codons in TGM1 may be due to the deamination of 5-CpG dinucleotides. In conclusion, this study expands the TGM1 mutation spectrum and represents the largest report of TGM1 mutations.

Divergent genotype is associated with type 2 diabetes in the Mongolian population. *Z. Odgerel[1], H. S. Lee[1], E. Narnygerel[2], N. Sambuughin[3], S. Ganbold[4], K. Altaisaikhan[2], L. G. Goldfarb[1]* 1) Clinical Neurogenetics Unit, NINDS/NIH, Bethesda, MD; 2) Diabetes Center, School of Medicine, Health Services University of Mongolia, Ulaanbaatar, Mongolia; 3) Department of Anesthesiology, Uniformed Services University of the Health Sciences, Bethesda, MD; 4) National Center of Forensic Sciences, Ulaanbaatar, Mongolia.

We conducted an association study to determine whether gene variants commonly found to be associated with type 2 diabetes (T2D) in large mixed populations or homogeneous populations living in various geographic regions are shared. For this purpose, we selected the Mongolian population of North-Eastern Asia that had experienced limited genetic admixture and environmental variability over about 1000 years. T2D morbidity in this population grew dramatically in recent years: a survey conducted by the WHO has established the prevalence of T2D at 3.2% and impaired glucose tolerance at 9.2%. Most significantly, there has been a 5-time increase in T2D prevalence within the past decade. In 80% of T2D patients the disease is poorly controlled, and the frequency of complications such as neuropathy was at 56.4%, retinopathy at 37.8%, and nephropathy at 65.6%. We examined the frequency of the T2D risk alleles at 11 polymorphic sites of 6 diabetogenic genes, PPARG, SLC30A8, TCF7L2, UCP2, KCNJ11, and ABCC8 in 211 patients diagnosed with type 2 diabetes and 196 non-diabetic Mongolian nationals from the city of Ulaanbaatar, Mongolia. Significant association was established with rs1799857 in the ABCC8 gene (OR = 1.52; P = 5.5x10-3) and rs660339 at the UCP2 gene (OR = 1.35; P = 2.5x10-2), while the variations in other genes failed to replicate associations repeatedly demonstrated in European large scale collaborative studies. These results, if confirmed on a larger sample size and in other populations with rapidly increasing T2D incidence, point to the existence of population-specific set of genes associated with the risk of T2D and the possibility that therapeutic regimens established in one population may not be as effective in other populations carrying a different T2D risk genotype.

Association of Interferon Regulatory Factor 5 (IRF5) polymorphisms with Systemic Sclerosis (SSc). *P. Gourh, S. Assassi, G. Paz, Y. Salehi, S. K. Agarwal, F. K. Tan, M. D. Mayes, F. C. Arnett* Division of Rheumatology, Department of Internal Medicine, University of Texas-Medical School, Houston, TX.

Background: Type-I interferon (IFN) signature has been shown to be the hallmark peripheral blood gene expression pattern in lupus (SLE). More recent studies have also noted a similar type-I IFN signature in systemic sclerosis (SSc). The transcription factor interferon regulatory factor 5 (IRF5) is a component of this IFN-gene expression signature and regulates the expression of other genes involved in immune responses. The purpose of this work was to investigate the possible association between IRF5 polymorphisms with SSc. **Methods:** We performed SNP genotyping for 3 SNPs on IRF5 gene using the Taqman Assay in 1,391 Caucasian, African-American, and Hispanic SSc patients along with 1,027 race-matched controls. All SSc patients fulfilled ACR criteria or had at least 3 of the 5 CREST features. Chi-square and logistic regression analyses were used for statistical comparisons. Illumina Human-REF8 arrays were used for peripheral blood gene expression analysis. **Results:** After HWE verification and correcting for multiple testing, two SNPs showed significant association with white SSc patients. The TT genotype for the SNP rs2004640 had a frequency of 34.5% in white SSc patients as compared to 27.1% in white controls which was statistically significant. Logistic regression analysis (*Additive model*) controlling for gender and race showed that the TT genotype was an independent risk factor for SSc {p=0.0004;OR=1.56 & 95% CI:1.3-2.0}, including anti-topoisomerase-I antibody positive SSc and SSc with fibrosing alveolitis. IRF5 was the most highly differentially expressed gene based on the rs2004640 SNP genotypes in peripheral blood arrays of SSc patients (p=1.39×10-5). **Conclusion:** These data suggest an important role of this IRF5 polymorphism in susceptibility to SSc. The TT genotype causes a splice variant containing exon IB whereas the GG genotype contains exon IA and exon IC. This IRF5 polymorphism leading to this isoform, upon stimulation, may facilitate expression of genes encoding type-I IFNs and other proinflammatory cytokines, thus increasing the risk for development of SSc.

Genome-wide linkage analysis for congenital heart defect risk reveals modifier loci of cardiac transcription factor Nkx2-5. *J. Winston, J. Erlich, C. Green, P. Jay* Dept Pediatrics, Washington Univ, St Louis, MO.

There is currently no logical explanation for the variance in congenital heart defects observed in human babies. Animal model and family studies have revealed that cardiac transcription factor NKX2-5 is a core regulator in heart morphogenesis. While cardiac development has been systematically characterized, it is unclear how NKX2-5 and other major genes fit into pathways that lead to naturally occurring heart defects. Heterozygous mutations in NKX2-5 increase risk for a broad spectrum of heart defects including atrial and ventricular septal defects (ASDs and VSDs) and aortic coarctation in both humans and mice. In this study Nkx2-5+/- haploinsufficient mice provide a model of heart defect risk variance to identify genes and their interactions that affect mouse cardiac developmental pathways. F1 hybrid progeny of Nkx2-5+/- C57Bl/6 mice crossed to FVB/N or A/J strains result in a nil or rare incidence of defects indicating that genomic homozygosity affects risk. Defects are recovered in F2 progeny of inter- and parental backcross backgrounds further validating the presence of alternative genomic risk loci. To identify QTL which modify the affect of Nkx2-5+/-, neonatal hearts of 2,100 F2 Nkx2-5+/- mice were dissected and serially sectioned to diagnose defects. A total of 320 heart defects have been identified with the vast majority being VSDs and ASDs. Genome wide linkage analysis for VSD susceptibility loci revealed a significant main effect locus on chromosome 6 (LOD = 3.1) in the initial F2 scan. Outcrossing Nkx2-5+/- mice to alternative strain, A/J, resulted in different defect yields. Through combined cross analysis, susceptibility loci specific to C57Bl/6 mice were found to be significant on chromosomes 4,10 and 11 (LOD= 3.6, 4.7 and 3.3). In addition, novel epistatic interactions were identified between loci on chromosomes 3 and 13. Genes within 25cM of these loci were prioritized based on gene expression and ontology data. The results in this study demonstrate that mechanistic insight can be gained through the description of small effect genes responsible for incomplete penetrance and pleiotropy commonly observed in complex disease.

A novel, autosomal dominant, Pseudoxanthoma Elasticum-like phenotype in a five-generation family. *M. L. P. Robert[1], O. M. Vanakker[2], L. Costrop[2], A. de Paepe[2], L. Schurgers[3], R. Florijn[4], F. M. Pope[5], M. James[6], S. Tomkins[6], E. Young[7], S. Ellard[7], P. D. Turnpenny[1]* 1) Clinical Genetics, Royal Devon & Exeter NHS Trust, Exeter; 2) Center for Medical Genetics, Ghent University, Ghent; 3) VitaK & CARIM, University of Maastricht, Maastricht; 4) The Netherlands Ophthalmic Research Institute, Amsterdam; 5) Northwick Park Health Institute, London; 6) South Western Regional Genetics Service, Bristol; 7) Molecular Genetics, Royal Devon & Exeter Hospital, Exeter.

Pseudoxanthoma elasticum (PXE) is an autosomal recessive (AR) progressive disorder of elastic fibres characterised by dermal, ocular and vascular lesions, due to mutations in ABCC6, an ATP-binding cassette transporter, in ~80% of cases. Autosomal dominant (AD) inheritance is very rare and usually due to pseudodominance (Plomp et al. 2004). We studied 17 individuals from a 5-generation family with PXE-like skin manifestations. Many subjects also suffer premature claudication pain and ischaemic heart disease but they do not have typical PXE ophthalmic signs. Electron microscopy analysis of skin was normal except in one patient who had fragmentation and clumping of elastic fibres with no evidence of calcification. Sequencing of ABCC6 was negative and linkage to this locus excluded. Vanakker et al (2007) reported GGCX mutations in 3 families with an AR PXE-like phenotype and vitamin K-dependent coagulopathy. Our family has no history of abnormal bleeding, clotting assays were normal in one affected individual, and sequencing of GGCX and VKORC1 was normal. Immunohistochemistry of lesional skin tissue showed disturbance in the gamma-carboxylation of vitamin K-dependent mineralization inhibitors, particularly matrix gla protein (MGP). This suggests involvement of a related pathway as in the PXE-like phenotype of Vanakker et al (2007). Genome-wide linkage results, and candidate gene sequencing, are awaited. This 5-generation family with a PXE-like phenotype demonstrates unequivocal AD inheritance. We believe this is a previously unreported clinical and genetic entity, the pathogenesis of which may provide new information on the molecular pathways involved in PXE and related disorders.

Overexpression of Targeting Protein for Xklp2 Affects Mitotic Kinase Aurora A Stability and Cell Cycle Progress in Melanoma Cells. *B. Li[1,2], M. Dalla Palma[1], Y. Wang[1], G. Xu[1], K. Smalley[2], M. Herlyn[2], K. Nathanson[1]* 1) Department of Medicine and Department of Pathology, University of Pennsylvania School of Medicine, philadelphia, PA; 2) The Wistar Institute,Philadelphia,PA.

Amplification and overexpression genes have been demonstrated to be involved in the development and progression of cancer. Array based comparative genomic hybridization analysis of melanoma cell lines and tumors has identified targeting Protein for Xklp2 (TPX2) as the most commonly amplified gene on 20q11.21, a region of frequent amplification in melanoma. Gene expression profiling studies also have confirmed that TPX2 is overexpressed, suggesting a previously unknown role in the development of melanoma. TPX2 is mainly expressed in G2/M, as a microtubule-associated protein participating in mitosis apparatus-bipolar spindle assembly and regulation of centrosome replication. TPX2 also activates and targets the mitotic kinase Aurora A to spindle pole. Whereas the physiological functions of TPX2 are well understood, consequences of aberrant TPX2 expression in tumor cells remain elusive. We have used shRNA to knockdown TPX2 to define its associated phenotype. With 80% knockdown of TPX2 in 293T cells, we found dramatic downregulation of Aurora A. We also have studied knockdown in melanoma cell lines overexpressing TPX2. Cell cycle profile analysis has been performed on TPX2shRNA virus-transduced melanoma cells. Preliminary data suggest that TPX2 shRNA depletion delays melanoma cell cycling due to marked G2/M arrest. IHC of TPX2 in melanomas suggests that expression increases with progression from in situ to metastatic disease. Taken together, these data suggest that TPX2 overexpression could promote cell cycle progression via aberrantly regulating mitotic apparatus assembly and Aurora A kinase activity and is important for tumor progression.

A Genetic Model for IRF5, Autoantibodies, and Interferon Alpha in the Pathogenesis of Systemic Lupus Erythematosus. *T. B. Niewold[1], J. A. Kelly[2], S. N. Kariuki[1], K. Thomas[2], D. Walker[2], S. Kampf[2], J. T. Merrill[2,3], M. E. Alarcón-Riquelme[4], J. A. James[2,3], T. J. Vyse[5], R. P. Kimberly[6], J. C. Edberg[6], P. M. Gaffney[2], K. L. Moser[2], M. K. Crow[7], J. B. Harley[2,3,8]* 1) Section of Rheumatology, University of Chicago, Chicago, IL; 2) Oklahoma Medical Research Foundation, Oklahoma City, OK; 3) Department of Medicine, University of Oklahoma, Oklahoma City, OK; 4) University of Uppsala, Uppsala, Sweden; 5) Imperial College, London, UK; 6) University of Alabama at Birmingham, Birmingham, AL; 7) Mary Kirkland Center for Lupus Research, Hospital for Special Surgery, New York, NY; 8) US Department of Veterans Affairs Medical Center, Oklahoma City, OK.

Objective: Interferon alpha (IFN-) has been implicated as a heritable risk factor for systemic lupus erythematosus (SLE). Variants of interferon regulatory factor 5 (IRF5) have been associated with SLE susceptibility, and we have recently shown that IRF5 risk variants are associated with increased IFN- only in a subset of patients defined by SLE-specific autoantibodies. **Methods:** We analyzed IRF5 genotype data from 1034 SLE patients and 989 controls of European ancestry, as well as 456 SLE patients and 679 controls of African American ancestry. Serum IFN- activity was measured using a reporter cell assay. **Results:** SLE-risk variants of IRF5 were associated with the SLE-specific autoantibodies anti-dsDNA (rs2004640) and anti-Ro (rs10488631) in European ancestry SLE patients. IRF5 haplotypes showed strong association with SLE only in 393 European patients with either anti-dsDNA or anti-Ro antibodies ($p=7.9x10^{-16}$), compared to 641 patients lacking anti-Ro and anti-dsDNA ($p=0.0019$). In African American SLE patients, association of IRF5 with SLE was only seen in the subgroup of patients with both anti-dsDNA and anti-Ro antibodies. In both ancestral backgrounds, IRF5 risk variants were associated with increased serum IFN- only in those patients with anti-dsDNA or anti-Ro antibodies. **Conclusions:** These data suggest an intriguing model of SLE pathogenesis, in which common IRF5 risk variants cooperate with rare SLE-specific autoantibodies to result in dysregulation of IFN- production and subsequent risk of disease.

Multiple Associations for Independent Single Markers within the HLA Region Confer Susceptibility to Psoriasis.

J. Raelson[1], L. Bennour[2], V. PInchuk[2], M.-T. Cheng[2], O. Gingras[2], P. Croteau[1], B. Stojkovic[2], A. Belouchi[1], S. Debrus[1], J.-F. Levesques[2] 1) Dept Genetics R&D, Genizon Biosciences, St Laurent, PQ, Canada; 2) Dept of Information Technology and Software Development, Genizon Biosciences, St Laurent, PQ, Canada.

A locus located in the MHC-HLA region of 6p21 at or near HLA-C has long been identified as being strongly associated with psoriasis. We have systematically examined single marker association and two marker haplotype association for all pairs of markers across the HLA region using a sample of 326 female and 310 male psoriasis patients and matching controls genotyped for 1,912 markers across 7.371 Mb. In addition to the well replicated association near HLA-C, we have detected multiple associations for both adjacent and widely separated interacting markers resulting in 5-10 fold increases in -log p-values for various 2-marker haplotypes compared to the maximum -log p-value for associations of either of the single markers alone. Highly significant associations for specific combinations of alleles for non adjacent markers that are in linkage equilibrium suggest complex patterns of HLA region gene interaction leading to susceptibility to psoriasis.

Goltz syndrome and X monosomy mosaicism: case report and literature review. *T. A. Zanolla[1,2], D. Polito[1], L. Kulikovski[2], L. V. Pereira[3], M. C. S. P. Cernach[1,2], A. B. A. Perez[1,2]* 1) UNIMES, Santos, São Paulo, Brazil; 2) Centro de Genética Médica, UNIFESP, São Paulo, São Paulo, Brazil; 3) IB-USP, São Paulo, São Paulo, Brazil.

Goltz syndrome is a disorder characterized by involvement of the skin, skeletal, eyes and face. It is a X-linked dominant disorder. Females are heterozygous or mosaic for mutations in PORCN(Xp11.23). Live-born affected males are mosaic for mutations in that gene. It is presumed that non-mosaic hemizygous are not viable. Available data suggest that neither the mutation nor level of mutated X-chromosome inactivated correlates with severity of the phenotype. All females with deletions in PORCN have skewed X-chromosome inactivation, whereas most females with point mutations have random X-chromosome inactivation (Grzeschik et al., 2007; Wang et al., 2007). We describe a 22 month-old female born from a non consanguineous young couple. Delivery was term by normal childbirth. Birth weight was 2,315 g (50th centile), height 46 cm (5th centile) and OFC 32 cm (5th centile). At 22 months she presents asymmetric facies, low set ears, convergent strabismus, up-slanting fissures, narrow nasal bridge, broad nasal tip, notched nasal alae, pointed chin, bilateral cleft lip and palate, hypoplasia of teeth,sparse and brittle hair with areas of alopecia, skin atrophy, dystrophic nails, ectrodactyly and syndactyly of hands and syndactyly between 3 and 4 toes of left foot, clitoral hypoplasia and minor labial hypoplasia. There is no clinical signs of Turner syndrome. G-banding karyotype from lymphocytes cells was 46,XX(35%)/45,X (65%. There is no case reported relating the association of Goltz syndrome and monosomy X mosaicism. We expect a mild phenotype as observed in liveborn males. All males reported to date are mosaic for mutations in PORCN and are generally more mildly affected than females, but there is no typical phenotype. The evaluation of which chromosome harbor the mutation and of the pattern of X inactivation may clarify embryonic period of protein expression.

Prenatal microarray analysis in an apparently balanced de novo translocation: a counseling dilemma. *V. Jobanputra[1], A. Shanmugham[1], S. Brown[2], A. Sobrino[1], O. Nahum[1], B. Levy[1], D. Warburton[1]* 1) Columbia University Medical Center, New York, NY; 2) University of Vermont, Burlington, VT.

We saw a 38 year old women, G4P1, in our Genetics Clinic to discuss fetal chromosome results showing an apparently balanced de novo translocation 46,XY,t(2;13)(q37;q32). The patient had an amniocentesis due to her maternal age and a two vessel-cord on level II sonogram. Parental chromosome studies were normal. The family elected to have microarray analysis on the fetal material, with the understanding that it might or might not be helpful. SNP oligonucleotide microarray analysis (SOMA) revealed a 384kb duplication at the 2q37.3 breakpoint and a 564 kb at the 13q32.3 breakpoint. While deletions of the 2q37 region have been reported in patients with the Albright Hereditary Osteodystrophy - like phenotype, a phenotype associated with the duplication of this region has not been described. The duplicated region on chromosome 13q contains the ZIC2 gene. Haploinsufficiency of ZIC2 is likely to cause holoprosencephaly and the brain malformations seen in distal 13q deletion patients. Studies in vitro have suggested that overexpression of ZIC2 may also lead to brain malformations in the mouse. No human data on overexpression exist. To confirm the duplications, FISH was performed using a genomic DNA probe that included the ZIC2 gene as well as two additional BACs localizing to the proximal and distal ends of the duplicated 2q37 region. The ZIC2 probe hybridized to both the derivative chromosomes, confirming the duplication, but the signal pattern on the der(2) was at the terminal region. This hybridization pattern suggests an inversion on 13q with the inverted segment containing ZIC2 on the der(2q). Both copies of the proximal BAC on 2q hybridized to the der(13) and the distal BAC was found on both derivative chromosomes. This suggests that an inversion and duplication also occurred on 2q. The microarray results were conveyed to the patient, together with the news that it was still not possible to predict the pregnancy outcome. A fetal MRI of the brain was normal. The patient has elected to continue the pregnancy and is now at 38 weeks. Sonograms have reported normal fetal anatomy.

RAPID TARGETED SEQUENCING OF MULTIPLE HUMAN MITOCHONDRIAL GENOMES. *G. Ehret[1], M. Sosa[1], L. Li[2], P. Bouffard[2], K. Fredrikson[3], T. Harkins[3], A. Chakravarti[1]* 1) Center for Complex Disease Genomics, McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD, USA; 2) 454 Life Sciences, 1 Commercial Street, Branford, CT, USA; 3) Roche Diagnostics Corporation, Indianapolis, IN, USA.

Defects in mitochondrial biology are known to contribute to the pathology of many complex diseases. An excess of maternal inheritance has been observed for some complex diseases while for others the age-dependence of the phenotype is thought to involve degradation of mitochondrial function. Consequently, accurate and rapid sequencing of mitochondrial genomes is important so that its sequence alteration can be correlated with disease. Our protocol for mitochondrial genome sequencing is based on two steps. First, specific access to the mitochondrial genome is obtained by amplification of three overlapping fragments by long range PCR. Second, accuracy in sequence is obtained by sequencing the pooled fragments using the emulsion PCR based Roche/454 FLX sequencer. This process provides simplicity in handling and high coverage. The sequencing depth not only permits de novo assembly of the mitochondrial genome and reliable identification of mitochondrial sequence variants, but also determination of heteroplasmy at high accuracy. We have successfully completed sequencing of 50 mitochondrial genomes from the Yoruba (YRI) and CEPH (CEU) HapMap samples at different coverage depths (47 samples at ~200x coverage, 3 samples at 1,000x coverage). Across the mitochondrial genome, we observe a minimum coverage of ~400 and ~80 fold (for the ~1,000x and 200x coverage, respectively). The mitochondrial genomes were assembled de novo using a random subset of the total data (~20x coverage). Our amplification and purification strategy for mitochondrial DNA is specific since <1% of the sequence is of non-mitochondrial origin. Gel purification of the mitochondrial DNA amplicons did not improve sequence specificity. We observe an overrepresentation of amplicon blunt ends among the sequence reads. Sequence features of these genomes, as also compared to conventionally obtained sequence, will be presented.

Interleukin-1 Receptor Antagonist Gene Polymorphism in Egyptian Patients with Knee Osteoarthritis. *E. A. Toreih[1], F. M. Badr[1], M. H. Ghattas[2]* 1) Human Genetics & Cell Biology, Faculty of Mrdicine/Suez Canal University, Ismailia, Ismailia, Egypt; 2) Department of Medical Biochemistry, Faculty of Medicine, Suez Canal University, Ismailia, Egypt.

The pro-inflammatory cytokine, interleukin-1 (IL-1), has been implicated in the pathogenesis of several arthritic diseases. One of its natural inhibitors, IL-1 receptor antagonist (IL-1Ra), is a potent anti-inflammatory agent. The aim of the present study was to determine the influence of a polymorphism within intron 2 of the IL-1 receptor antagonist gene (IL-1RN) on the susceptibility to or severity of knee osteoarthritis (OA) among Egyptian patients. We genotyped 38 patients with primary knee OA and 38 healthy controls for IL-1RN variable number of tandem repeat (VNTR) polymorphism (for six different alleles), using polymerase chain reaction-based method. The heterozygote form A1/A*2 was the most predominant genotype among patients with knee OA compared with the controls (60.5% vs 31.5%, p= 0.012). The frequency and carriage rate of A2 allele was significantly higher in OA patients compared with the controls (frequency 38.2% vs 17.1%; carriage rate 68.4% vs 34.2%). Alleles A0, A4 and A5 were absent in the current study population. Frequency and carriage rate of A2 allele were associated with the severity of joint pain (p=0.016), but had no association with other clinical or radiological parameters of the disease. Our results suggest that IL-1RN gene polymorphism is associated with the occurrence of knee osteoarthritis. Whether the IL-1RN polymorphism makes a direct functional contribution to the pathogenesis of the disease or is acting as a marker for a linked gene needs to be investigated.

Bivariate genome-wide linkage scan for Traits BMD & AAM: Effect of Menopause on Linkage Signals. *Z. X. ZHANG[1], S. F. Lei[2], F. Y. Deng[2], F. Zhang[1], Y. J. Liu[2], R. R. Recker[3], H. W. Deng[1, 2, 3, 4]* 1) School of Life Science and Technology, Xi'an Jiaotong University, China; 2) Departments of Orthopedic Surgery and Basic Medical Science, School of Medicine, University of Missouri-Kansas City, USA; 3) School of Medicine, Creighton University, USA; 4) College of Life Sciences, Hunan Normal University, China.

Osteoporosis is an age-related systemic skeletal disease, characterized by low bone mineral density (BMD). Low BMD is closely associated with later age at menarche (AAM). Our previous bivariate genome-wide linkage analyses (GWLAs) between BMD and AAM were performed in 2522 Caucasian female subjects of European origin, including both pre- and post- menopausal females. However, the menopause causes a dramatic bone loss in postmenopausal females. This may introduce some confusing effect on the bivariate GWLA for BMD & AAM. To address the menopause effect on the estimation of genetic effects shared by BMD & AAM, we performed further bivariate GWLAs separately in two subgroups: 1462 pre- and 1122 post-menopausal Caucasian females. The phenotypes of BMD were measured by Hologic Dual-energy X-ray (DXA) scanners (Hologic Inc., Bedford, MA, USA). Adopting genome-wide thresholds corrected for multiple testing, we found 6, 2, and 2 significant genomic regions shared by spine BMD & AAM, hip BMD & AAM and UD BMD & AAM, respectively in pre-menopausal group, but we did not found any significant or suggestive linkage signals in post-menopausal group. The linkage signals are much stronger in pre-menopausal group than in other groups: post- menopausal females and total females. For example, the linkage LOD for hip BMD & AAM is as high as 5.1 in pre-menopausal females, but only 0.07 and 0.31 in post-menopausal females and total females, respectively. The results suggest that the estimation of shared genetic factors between BMD & AAM may be introduced some noise signals when including postmenopausal female, and more targeting genomic regions can be found if the female subjects were classified properly according to their menopausal status.

MEDICAL SEQUENCING OF 11 HYPERTENSION GENES IN THE EXTREMES OF THE BLOOD PRESSURE DISTRIBUTION*. *K. H. Nguyen, G. B. Ehret, A. Chakravarti* Center for Complex Disease Genomics, McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD, USA.

The genetic pathophysiology of essential hypertension (EH), despite its significant genetic component, still remains unknown. Although many linkage and association studies are underway, we wished to directly assess the contribution of DNA sequence variation at specific genes to EH. We examined 560 individuals randomly sampled from the ~15% extremes of the systolic blood pressure (SBP) distribution in GenNet, equally divided into African & European American (AA & EA), male & female samples. We sequenced 10 monogenic hypertension syndrome genes (CYP11B1, CYP17A1, HSD11B2, NR3C1, NR3C2, SCNN1A, SCNN1B, SCNN1G, WNK1, and WNK4) and angiotensin (AGT). For each, we sequenced all exons, 2kb up- & down-stream of the first & last exon, and all conserved intronic, 5& 3 elements beyond the 2kb boundary using bidirectional dideoxy Sanger sequencing. Sequencing quality was high with an average variant call rate of 93% and a replicate consistency of 99.8%. For each individual, we generated 152kb of sequence or ~85Mb in total yielding 2,691 variants (2,529 SNPs & 162 indels): ~1350 variants were unique to AA, ~610 to EA and ~730 were common. We observed a ratio of 4 novel:known (dbSNP, r128) substitutions and 14 for indels, higher than in previous studies. The overall distribution of sequence changes varies across genes but shows no pattern in the top and bottom 15%tiles. Individual genes do show differences with non-synonymous coding sequence changes that we are correlating with predicted functional effects (using the computer programs SIFT and PolyPhen). Interestingly, substitutions are enriched for WNK1 and AGT in the lower 15%tile and indels are concentrated largely in WNK1 (29% of total) and NR3C2 (45% of total). These types of sequence patterns are being assessed with respect to the measured BP values. This study demonstrates the value of direct DNA sequence data in studying complex traits and diseases. *Resequencing services: the J. Craig Venter Inst. and the Univ. of Washington, Dept of Genome Sciences, under U.S. Fed Gov contract #N01-HV-48196 and N01-HV-48194 from the NHLBI.

Hybrid Capture and Deep Resequencing of Stromally Contaminated Lung Adenocarcenoma. *K. Cibulskis[1], C. Sougnez[1], J. Maguire[1], S. Fisher[1], L. Ambrogio[1], M. Meyerson[2], E. Lander[1], S. Gabriel[1]* 1) Broad Institute of MIT and Harvard, Cambridge, MA; 2) Dana Farber Cancer Institute, Boston, MA.

Targeted resequencing of exons using capillary sequencing has successfully been used to find somatic alterations in cancer DNA, leading to the discovery of genes significantly associated with cancer. However, stromal contamination of tumor DNA makes the discovery of mutations even more difficult, and could lead to an underestimation of mutation frequency. One such large-scale capillary sequencing project is the Tumor Sequencing Project (TSP), which sequenced 602 genes across 188 lung adenocarcinoma samples. In the TSP, the least pure 30% of samples sequenced contained only 8% of the total mutations. We sought to determine the utility of deep sequencing, provided by next generation sequencing technologies, in discovering additional mutations in samples ranging in purity from ~ 30% to 90% by pathology review.

To explore this, we have performed deep sequencing (to an average 100-fold coverage) using the Illumina GAII platform on 24 tumor/normal pairs which have previously been sequenced via capillary sequencing. We targeted 75 genes previously found to be significantly mutated in this tumor type. Instead of PCR, a solution-based capture technique was used for target enrichment. We discuss the specificity and sensitivity of this approach, as compared to the current gold standard of PCR amplification and capillary sequencing, and the impact of deep sequencing on the overall frequency of mutation within these genes.

Single-molecule analysis of DNA methylation in induced pluripotent stem cells. *J. Deng[1], B. Xie[2], E. Leproust[3], D. Egli[4], N. Maherali[5], I. H. Park[6], K. Eggan[4], K. Hochedlinger[5], G. Daley[6], Y. Gao[2], K. Zhang[1]* 1) Dept. of Bioengineering, UCSD, La Jolla, CA; 2) Virginia Commonwealth Univ, Richmond, VA; 3) Agilent Technologies Inc, Santa Clara, CA; 4) Harvard Univ, Cambridge, MA; 5) Mass. General Hospital, Boston, MA; 6) Children's Hospital, Boston, MA.

DNA methylation is one of the primary epigenetic regulatory mechanisms involved in normal developmental processes and many human diseases including cancers. Rapid advances in DNA sequencing have catalyzed the development of new methods for characterizing DNA methylation in an unprecedented scale and resolution. Here we present a targeted bisulfite sequencing method, called cpgMIP, for digital quantitation of DNA methylation at the single-nucleotide resolution. In this method we synthesized libraries of long oligonucleotides on programmable microarrays, and enzymatically converted them into Molecular Inversion Probes (MIPs) targeting CpG islands (CGIs) in the human genome. CGIs were specifically captured from the bisulfite converted genomic DNA, and sequenced with next-generation DNA sequencers. cpgMIP dramatically reduces the size of sequencing targets and results in a >100x improvement in efficiency compared with whole genome bisulfite sequencing. We used cpgMIP to characterize the patterns of DNA methylation in the reprogramming of human fibroblasts into pluripotent cells. A total of 30,000 probes were synthesized for capturing 83% of non-repetitive CGIs in human chromosome 12 and 20, both were enriched for genes differentially expressed between fibroblasts and pluripotent cells. A capturing specificity of 100% was achieved with these probes. We characterized the methylation patterns in three pairs of fibroblasts and induced pluripotent stem cells reprogrammed with different sets of transcription factors, as well as a fourth pair of fibroblasts and pluripotent cell lines generated by somatic cell fusion. Functional CpG sites were identified through comparison with gene expression profiles. In summary, cpgMIP is a highly efficient method for accurate and large-scale characterization of DNA methylation. This method will help achieving a deeper understanding of DNA methylation in transcriptional regulation.

Updating the diagnostic decision tree for recessive hereditary sensory and autonomic neuropathies (HSAN). *C. Oddoux[1], H. Ostrer[1], F. Axelrod[2]* 1) Div Human Genetics, NYU Langone Medical Center, New York, NY; 2) Dysautonomia Diagnosis and Treatment Center, NYU Langone Medical Center, New York, NY.

Background: Recessive HSANs are a rare group of hereditary disorders characterized by dysfunction of the sensory and autonomic nervous systems. Classification of various subtypes remains incomplete and is continuing to evolve in part due to variable presentation, overlap of phenotypes, and the lack of consistently used objective measures of autonomic and sensory function. HSAN type 3 (familial dysautonomia) is the most common of the group, followed by HSAN type 4 (congenital insensitivity to pain), HSAN type 2 (HSN2), and HSAN type 5. Mutations in several genes have been found to be responsible for some of these phenotypes.

Methods: Clinical evaluation of sensory and autonomic function was performed on a large series of HSAN patients and correlated with mutation and sequence information in the IKAP, NTRK1, HSN2 and NGFbeta genes. Clinical assessments included response to intradermal histamine, pain, temperature, taste, positional sensation, corneal, gag and deep tendon reflexes, postural hypotension, erythmatous blotching, presence of fungiform papillae, gastrointestinal motility, tearing, anhydrosis and Charcot joints. These were further correlated to a meta-analysis of published studies of similar patients and these genes and to genomic information about the genes available in public genome databases. The data were used to derive a clinical evaluation decision tree to assist with classification of patients and to inform subsequent treatment, genetic testing, and counseling.

Conclusions: The results suggest that it is possible to use systematic non-invasive clinical evaluations to determine which patients have a high likelihood of having mutations in the IKAP, NTRK1, and HSN2 genes, thereby enabling efficient diagnosis, initiation of treatment and delineation of patients most likely to benefit from genetic testing for enhanced family planning. The results also reveal a large group of patients that do not fall into any of these categories suggesting that mutations in other genes, or *de novo* events may be responsible for their phenotypes.

Molecular cytogenetic and microarray characterization of a de novo der(Y)t(X;Y)(q27.1;q12) in a male with mental retardation, hypotonia, intractable seizure disorder and dysmorphic features. *A. Iglesias[1], M. Macera[2], P. Cotter[3], F. Cohen[2], J. Breshin[2], A. Babu[2]* 1) Dept Pediatrics, Div Genetics, Beth Israel Medical Ctr, New York, NY; 2) Dept Medicine, Div Molecular Medicine and Genetics, Wyckoff Heights Medical Ctr, Brooklyn, NY; 3) CMDX Laboratory, Irvine, CA.

The patient is a 12 year-old boy with seizure disorder, hypotonia and mental retardation. A brain MRI during infancy showed thinning of the corpus callosum. He was born full-term via c-section. Hypotonia, poor suck, vomiting and poor weight gain were noticed after birth. Gastroesophageal reflux was diagnosed. Floppiness and developmental delay were present early on. Seizures developed at 16 months. On exam, he was poorly reactive and non-interactive. A wide nasal bridge, full lips, short philtrum, macroglossia, high palate and abnormal ears were noted. Scoliosis and generalized joint contractures were present bilaterally. He has tapering fingers. Axial hypotonia and distal spasticity were present. Mydriasis and slow pupillary responses and soles hyperemia were observed bilaterally. Cytogenetic analysis revealed a 46,X,der(Y)t(X;Y)(q27;q12) karyotype in peripheral blood cells. Microarray-CGH showed a gain in copy number in the distal regions of Xq, (Xq27.2Xqter) encompassing 15.3Mb. Additional microarray studies at a separate facility, confirmed the duplication with an approximate size of 13.7 Mb from Xq27.2qter. Gene analysis of the MECP2 and LICAM1 genes also confirmed the duplication. When wcp X was applied, signal was visible at the distal q arm of the der (Y), corresponding to the translocation. The signal observed on the der(Y) appeared to be greater than the corresponding duplicated Xq27.2qter and is being investigated. The probands father had a normal 46,XY karyotype, establishing the de novo origin of this derivative chromosome, with the duplication and translocation most likely occurring during meiosis II. Duplications of the distal Xq encompassing the MECP2 with an average size of 2Mb have been recently reported with a related phenotype. To our knowledge, the size of this rearrangement is much larger than the previous cases and might help to elucidate our knowledge of these conditions.

Molecular cloning of LOC348751 and its association with T2D in Pima Indians. *T. Guo, Y. Dong, J. Dooley, R. Hanson, K. Sayuko, C. Bogardus, L. Baier* PECRB, NIDDK/NIH, Phoenix, AZ.

A genome-wide association study using the Affymetrix 100K chip was done in Pima Indians to identify susceptibility genes for type 2 diabetes mellitus (T2D). SNPs were initially genotyped in 895 case/control subjects for early-onset T2D, among which 482 were discordant siblings. SNPs nominally associated with T2D under both a general and within family analysis were prioritized for follow-up studies. One prioritized SNP, rs10497841, was followed-up by genotyping in a population-based sample of 3501 full-heritage Pima Indians (1561 with T2D), where its association with T2D was confirmed ($p=0.004$ and 0.04 for general and within family, respectively, adjusted for age, sex, and birth-year). In addition, among 372 subjects who had undergone metabolic testing for predictors of T2D when they were non-diabetic, the risk allele for T2D was associated with reduced insulin-mediated glucose uptake (ie- insulin resistance) during a hyperinsulinemic, euglycemic clamp (adjusted $p=0.008$). The risk allele for T2D and insulin resistance (C) is common in Pima Indians (frequency= 0.85), but is less common in other ethnic groups (frequency in Caucasians and Africans= 0.34 and 0.12 respectively). Further fine mapping using 31 SNPs in this region suggested that the associated variant is within a 300 kb haplotype block that includes 3 reference genes, (FLJ38973, C2orf60 and C2orf47) and a non full-length transcript LOC348751. Among these genes, only LOC348751 had a predicted conserved functional domain that could potentially be involved in a metabolism. This partial transcript was highly expressed in human insulin action tissues such as skeletal muscle and liver. We used RACE to identify the 5 and 3 ends of this transcript and detected 2 transcripts, both predicted to have a formiminotransferase N-terminal conserved domain (FTCD-N). A homologous gene FTCD encodes formiminotransferase-cyclodeaminase which serves to channel one-carbon units from formiminoglutamate to the folate pool. We propose that LOC348751 may potentially confer risk of T2D and insulin resistance via its action in a folate metabolic pathway.

Mutation in ITPR1 underlies an ataxic movement disorder in both mice and humans (SCA15). *J. van de Leemput[1,2], J. Chandran[3], M. A. Knight[4], L. A. Holtzclaw[5], M. R. Cookson[1], H. Houlden[2], J. Hardy[6], E. M. C. Fisher[2], J. T. Russell[5], H. Cai[1], A. B. Singleton[1]* 1) NIH/NIA, USA; 2) UCL/IoN, UK; 3) University of Edinburgh, UK; 4) NIH/NHGRI, USA; 5) NIH/NICHD, USA; 6) UCL/Reta Lila Weston Institute, UK.

A severe autosomal recessive movement disorder was observed in mice used within our laboratory. Disease-onset is around P14, characterized by ataxic movement, loss of balance, truncal torsions and tonic/ tonic-clonic seizures. The disorder is progressive and lifespan is 3-4 weeks. Interestingly, heterozygotes do not display any of the disease-related features. Through linkage and sequence analysis the disorder was shown to be caused by a homozygous in-frame 18bp deletion in the gene Itpr1, encoding inositol 1,4,5-triphosphate receptor type 1. Itpr118 leads to a decrease in the normally high level of ITPR1 expression in cerebellar Purkinje cells, as demonstrated by immunohistochemistry and western blot analysis. In addition, a gene expression study in cerebellar tissue of Itpr1 mice was carried out to study the molecular mechanism that regulates calcium balance in the absence of ITPR1. Spinocerebellar ataxia 15 (SCA15), a human late-onset autosomal dominant disorder, maps to the genomic region containing ITPR1; however, no causal mutations had been identified. Sequence analysis of the coding exons of ITPR1 and high density genome-wide SNP genotyping were performed concurrently to test the hypothesis that mutation at ITPR1 may be the cause of SCA15. Sequence analysis failed to show any alterations segregating with disease. However, genome wide SNP genotyping metrics showed heterozygous deletion of the 5 part of the ITPR1 gene, encompassing exons 1-10, 1-40 or 1-44 in three studied families, to underlie SCA15. Moreover, western blot analysis showed a decrease in ITPR1 protein levels in EBV immortalized lymphoblasts of affected, but not unaffected, members of the original SCA15 family. In addition, classical sequencing techniques were applied to a large cohort of SCA families to identify distinct ITPR1 mutations, and assess gene dosage of ITPR1 in order to estimate disease prevalence.

ClinSeq: A pilot for the development of high-throughput genomic sequencing as a tool for translational genomics. *L. Biesecker[1,2], F. Facio[1], J. Teer[1], R. Cannon[3], T. Finkel[3], A. Remaley[4], G. Bouffard[2], J. Mullikin[1,2], J. Shendure[5], E. Green[1,2], NISC Comparative Sequencing Group* 1) NHGRI, NIH, Bethesda, MD; 2) NISC, Rockville, MD; 3) NHLBI, NIH, Bethesda, MD; 4) NIH Clinical Center, Bethesda, MD; 5) Univ Washington, Seattle.

ClinSeq is a pilot to develop methods and approaches for whole-genome sequencing to be used in clinical research. We will study data generation, archiving, access and sharing, sequence variant detection, determination of pathogenicity, returning results to the subjects, and informed consent. The study will initially sequence about 400 genes relevant to atherosclerotic heart disease in 1,000 patients. When advances permit, the study will be expanded to the entire exome or genome. At submission, we have recruited more than 400 subjects and have generated more than 930,000 capillary sequencing reads of ~3,500 genomic target sequences, ~275 Mbp of bidirectional genomic sequence. The accuracy of the sequencing was measured using 9 HapMap DNA samples, showing a 4% discordance rate and 90% sensitivity over a sample set of 1,256 HapMap SNPs that overlap the ClinSeq gene list. An additional 1,138 novel variants, relative to dbSNP, were detected across 201 subjects. We detected pathogenic variants in known disease genes, including LDLR, APOB1, and others. Several of these were validated in a CLIA lab and returned to the subjects, with genetic and medical counseling. We are also developing molecular inversion probe capture target selection with solid phase sequencing. When compared to the PCR/capillary sequence data, for a variant called in any patient (~3,100-3,700 positions) genotypes were called at 42% of these positions, and 98% of the time, the calls agreed. These preliminary results demonstrate that high throughput sequencing can be applied to clinical research, that scientifically and clinically useful data can be derived from such a study, that the return of appropriate results is practical and ethically appropriate, and that it is feasible to consent clinical research subjects to a whole-genome sequencing study. The clinical research opportunities made possible by high-throughput clinical genomics will be discussed.

MOHR SYNDROME: CASE REPORT AND CLINICAL DISTINCTION BETWEEN ORO-FACIAL-DIGITAL SYNDROMES TYPE I AND II. *P. Nicola, G. Machado, Z. Lumack, J. Sanches, D. Brunoni, A. Perez* Dept Morphology, Univ Federal de Sao Paulo, Sao Paulo, Brazil.

The Oral-Facial-Digital Syndrome type II (OFD II) is an inherited condition phenotypically characterized by ocular hypertelorism, low nasal bridge, high-arched palate, lobulated tongue, syndactyly, brachydactyly and polydactyly. It was first described by Otto L. Mohr in 1941. Many types of Oral-Facial-Digital Syndromes were reported and the last one was OFD type XIII. All of them present oral, face and digits malformations. The OFD II presents conductive hearing loss and autosomal recessive inheritance. We reported a boy, the third child of non-consanguineous couple, that was born at full term, by cesarean delivery, birth weight was 3115g (p25), length 47cm (p3), and OFC 34cm (-1dp). Multiple congenital anomalies were noted including a non-communication hydrocephaly, right parietal brain cyst, hypertelorism, strabismus, low nasal bridge, triangulate mouth, hypertrophied oral frenula, cutaneous syndactyly between 3/4/5 fingers and also between 1/2 of right hand and partial duplication of right hallux. At the age of 1.7 years, the child presented weight 9450g (p<3), height 77cm (P3), OFC 44,5cm (p<3), microcephaly, delay of neurologic development and the bone age. This case report represents an OFD II due to clinical, radiological manifestation and also the central nervous system anomalies. As we have already reported the parents are non consanguineous. We classified this case on OFD type II syndrome based on phenotype, but there are clinical signs of the two principal forms of OFD syndromes, type I and II. There are few published cases of Mohr syndrome and we think that this case contributes to delineation of the syndrome. The correct definition of this heterogeneous group of syndrome is important also due to different pattern of inheritance.

Epigenetic silencing of LINE-1 retrotransposition events in human embryonic carcinoma cell lines. *J. L. Garcia-Perez[1], M. Morell[2], D. A. Kulpa[3], C. C. Carter[4], K. L. Collins[3,4], K. S. O'Shea[2], J. V. Moran[1,3,4]* 1) Human Genetics; 2) Cell and Developmental Biology; 3) Internal Medicine; 4) Cellular and Molecular Biology Program, University of Michigan Medical School, Ann Arbor MI.

Long INterspersed Element-1 (LINE-1 or L1) is an abundant retrotransposon that comprises ~17% of human DNA. The average human is estimated to contain ~80-100 active L1s, and L1 mobility (retrotransposition) continues to impact the genome in a myriad of ways. Despite these facts, how L1 retrotransposition is regulated remains unclear. Here, we have used engineered L1s equipped with an enhanced green fluorescent protein (EGFP) retrotransposition indicator cassette to assess L1 mobility in cultured embryonic carcinoma (EC) cell lines that naturally express both L1 RNA and the L1-encoded proteins. Our data demonstrate that certain EC cell lines (PA-1, 2102Ep, and 833KE cells) support L1 mobility, but that the resultant retrotransposition events undergo efficient epigenetic silencing either during or shortly after their integration. Remarkably, treatment of PA-1 cells containing silenced L1 insertions for 16 hours with the histone deacetylase inhibitors (IHDAC) trichostatin A, sodium butyrate, or valproic acid reverses epigenetic silencing, resulting in an approximate 20-fold increase in the number of cells expressing the retrotransposed EGFP indicator cassette. A similar trend in data also was obtained from experiments conducted with natural and synthetic mouse L1s and a zebrafish LINE-2 element. In contrast, control experiments demonstrated that an EGFP expression cassette delivered by either a Moloney murine leukemia virus vector or HIV is not efficiently silenced in PA-1 cells. Our data further suggest that L1 epigenetic silencing is tightly associated with the undifferentiated status of EC cells. In sum, we have shown that retrotransposition events derived from a cohort of non-LTR retrotransposons are efficiently silenced in various EC cell lines by a mechanism that likely involves the post-translational modification of histones. We speculate that the epigenetic silencing of de novo L1 insertions could represent a mechanism employed by the host to regulate retrotransposition.

FOLLOW-UP OF A MAJOR LINKAGE PEAK ON CHR 1 REVEALS MULTIPLE QTLS ASSOCIATED WITH ESSENTIAL HYPERTENSION: GENNET STUDY. *A. A. O'Connor[1], G. B. Ehret[1], A. Weder[2], R. Cooper[3], A. Chakravarti[1]* 1) Center for Complex Disease Genomics, McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD, USA; 2) Division of Cardiovascular Medicine, Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, MI, USA; 3) Department of Preventive Medicine and Epidemiology, Loyola University Stritch School of Medicine, Maywood, IL, USA.

Essential hypertension is a principal cardiovascular risk factor with a significant genetic component. In contrast to other complex genetic traits, recently published genome-wide association studies have, however, failed to identify significant and replicable genetic findings for essential hypertension. We have previously published a large genome-wide linkage scan in Americans of African and European descent using data from the GenNet network of the NHLBI-supported Family Blood Pressure Program. The most significant linkage peak was on chromosome 1q, spanning a region of 100cM. In this study, we have genotyped 1,569 marker SNPs under the linkage-peak in 2,379 individuals in order to identify genetic variants that may be associated with blood pressure (BP). Our analysis, using two different family-based association tests, identifies a significant number of variants associated with BP. Using diastolic blood pressure as a quantitative phenotype, we identify three variants, located in or near the GPA33, CD247, and F5 genes, yielding significant p-values after Bonferroni correction (nominal p = 2x10-5). Using systolic blood pressure, variants in GPA33, CD247 and REN (nominal p = 8.3x10-6) are significant. In summary, we show that a consequential follow-up of a linkage signal can help to discover variants with small genetic effects that will be missed by genome-wide association studies. This collection of SNPs will serve as a priority list for future investigations of BP variants on the human chromosome 1.

Combined molecular and clinical analysis in Fanconi Anemia patients: A powerful approach to patient assessment. *M. A. Johnson[1], N. N. Moghrabi[1], X. Zhu[1], M. J. Al-Dhalimy[1], S. B. Olson[1], M. Grompe[1], B. P. Alter[2], C. S. Richards[1]* 1) Dept Molec & Med Gen, MP350, Oregon Health & Sci Univ, Portland, OR; 2) National Cancer Institute, Rockville, MD.

Fanconi anemia (FA) is a rare (<1:100,000 live births) genetically heterogeneous chromosomal breakage disorder exhibiting either autosomal recessive or (very rarely) X-linked mode of inheritance. FA is characterized by specific birth defects, progressive bone marrow failure, and specific malignancies (myeloid leukemia and epithelial tumors) associated with genomic instability. Diagnoses of FA patients are routinely confirmed by the presence of chromosomal breakage and radial formation in lymphocytes or fibroblasts due to increased sensitivity to DNA cross-linking agents such as diepoxybutane (DEB) and mitomycin C (MMC). These cells are further characterized by complementation group analysis using retroviral transfection and rescue of cross-link sensitivity. Mutations in complementation group A gene (*FANCA*), account for approximately 65% of all FA cases, with mutations in *FANCC, FANCG, FANCE*, and *FANCF* contributing an additional 25%. In this study we demonstrate the clinical utility of our laboratory-developed comprehensive molecular FA analysis. We used this molecular analysis, to validate complementation group assignment for *FANCA, FANCC, FANCG*, *FANCE* and *FANCF*, and identified and characterized FA mutations. We used a clinical information survey tool to capture clinical patient data. Combining these data with our patient mutation information enabled a systematic approach to elucidation of genotype-phenotype correlations. Additionally, in our cohort of 48 confirmed FA patients we have identified 27 novel putative pathogenic mutations, all of which will be deposited in a public data base. These results validate the clinical utility of FA complementation group assignments, detail our FA genotype-phenotype study, and document the discovery of novel FA mutations.

Limb/pelvis-hypoplasia/aplasia syndrome (#276820): Report of a Brazilian patient and discussion of the differential diagnoses. *Z. D. N. M. Lumack[1], A. B. A. Perez[1], T. A. Zanolla[1], M. F. F. Soares[2], M. C. S. P. Cernach[1]* 1) Centro de Genética Médica (CGM), Universidade Federal de São Paulo (UNIFESP), São Paulo, Brasil; 2) Departamento de Radiologia e Diagnóstico por Imagem, Universidade Federal de São Paulo (UNIFESP), São Paulo, Brasil.

The limb/pelvis-hypoplasia/aplasia syndrome (LPHAS) - described by Al-Alwadi in 1985 - is a rare condition, that seriously affects the limbs and pelvic bones with autosomal recessive inheritance. We describe a newborn, first daughter of nonconsanguineous young couple, without teratogenic exposures during the uneventful pregnancy. Born mature, weight: 2535g (10th centile), length: 30cm (<3rd centile), head circumference: 32,5cm (<3rd centile). Physical examination: brachycephaly, telecanthus, microtia, depressed nasal root and broad tip, midfacial capillary hemangioma, posterior cleft palate and bifid uvula. Genitalia: Hypoplastic labia, anterior displacement of the anus. Limbs: contractures at the elbows and knees, mesomelic shortening. Hands: brachydactyly. Feet: total syndactyly of the first and second toes with medial deviation and nail hypoplasia. C-banded karyotype: 46,XX, without premature centromere separation. Abdominal ultrasound: pyelectasis; paranasal sinus computerized tomography: left choanal atresia. Skeletal radiographs: hypoplastic ulnae, hypoplastic radius on the right and absent radius on the left. Bilateral tibiae and fibulae agenesis, short femur on the right and short and bowed femur on the left. Sacrum hypoplasia, coccyx and iliac agenesis. She underwent a laparotomic exploration due to a peritonitis, developed septicemia and died with three months. No additional malformation was noted at autopsy. Multiple congenital anomalies involving the skeleton comprise a phenotypically and genetically heterogenous group of conditions that in some instances bring prompt difficulties toward the differencial diagnosis. In 2006, Woods et al considered the WNT7A gene, in 3p25.1, a candidate for causative mutations in the family described by them. Further evaluation is needed to determine to what extent this gene could be involved in the aetiology of LPHAS.

Gene variants associated with coronary artery disease presenting as sudden death. *K. L. Hamilton[1], E. Rampersaud[1], D. Gamal el-Iman[2], R. Faugue[2], E. K. Mont[4], N. Yeboah[1], R. J. Myerburg[2], E. Martin[1], N. H. Bishopric[2,3]* 1) Miami Institute for Human Genomics, University of Miami Miller School of Medicine; 2) Department of Medicine, University of Miami Miller School of Medicine; 3) Molecular and Cellular Pharmacology, University of Miami Miller School of Medicine; 4) Miami-Dade County Medical Examiner Department, Miami, FL.

Sudden cardiac death (SCD) is a complex trait with multiple environmental and genetic factors. Cardiac ion channel mutations have been reported in association with 1/3 of cases of autopsy-negative SCD in young persons; however the contribution of genetic factors to SCD in the setting of common acquired cardiovascular disorders including acute cardiovascular syndrome (ACS) is unknown. We hypothesized that common polymorphic variants in these same genes may be associated with the occurrence of SCD as the initial presentation of ACS. 131 cases of SCD with critical coronary narrowing and/or fresh thrombus, but no evidence of previous myocardial scarring, were identified retrospectively in white SCD cases collected from the Miami-Dade coroners office. 662 German white subjects from the KORA population served as controls. 1,536 SNPs were genotyped using a GoldenGate genotyping assay. Quality control (QC) measures included elimination of SNPs and samples with low call rates (<99%), and checks for Hardy-Weinberg disequilibrium and population stratification. Association with SCD was tested using age and sex-adjusted logistic regression analysis. Cases were younger than controls (52 vs. 61 yrs) and were mainly male (93%). No population substructure was detected in these samples. 1,403 SNPs passed our QC checks. 181 SNPs were associated with SCD ($p<0.05$). The most highly associated were several correlated SNPs in ANK2 on chromosome 4 ($p<0.001$), KCNQ1 on chromosome 11 ($p<0.001$), and KCNE1 on chromosome 21 ($p<0.001$). In a cohort of white patients with SCD as the initial presentation of ACS, our high-density candidate gene analysis preliminarily identified association with SNPs in several genes regulating I_{Ks}. Variability in I_{Ks} function may be a major modulator of susceptibility to cardiac arrest as the initial presentation of ACS.

Identifying copy number variations: A comparison of array platforms (Nimblegen vs Agilent), quantitative PCR and SNP using the Parkin gene in Parkinsons Disease. *K. Raiford[1], J. Deng[2], Y. Li[3], L. Nathanson[1], D. Casadeseus[1], S. Zuchner[1], J. Vance[1]* 1) Institute for Human Genomics, University of Miami, Miami, FL; 2) Department of Neurobiology, School of Medicine, Duke University, Durham, NC; 3) Department of Medicine, Duke University, Durham, NC.

The role of genomic copy number variation (CNV) in disease susceptibility is the focus of many recent studies of complex disorders. Multiple CNVs have been described in the parkin gene (PARK2). These are postulated to contribute to loss of function and subsequent Parkinsons disease. A number of methods have been developed to screen for CNVs in DNA, including quantitative PCR (qPCR), loss of heterozygosity (LOH), and array-based assays (high-density SNP, specific comparative genomic hybridization (CGH) arrays), each with different advantages and disadvantages. Further complicating the analysis of CNV is the existence of different statistical algorithms for calling variants. We initially screened 789 PD patient and 174 control individuals using qPCR then followed potential CNVs with two high density array platforms: Agilent 8-plex 15K CGH array, and NimbleGen 4-plex 385K CGH array. When available, affected family members were also evaluated to test for segregation of the CNV. Multiple putative CNV were identified with each of the assays but results were not always concordant across algorithms. To explore this we compared the efficiency of CNV detection, the sensitivity, and the overall reproducibility of these three methods. We also compared the properties of different analysis algorithms implemented in array analysis software CGH Analytics and Nexus. This study provides important, practical guidelines for molecular assays and statistical detection of CNV.

Diagnosis of biotinidase deficiency by full gene sequencing of the BTD gene. *M. Procter[1], C. Glezos[1], P. Dobrowolski[1], M. Pasquali[1,2], R. Mao[1,2]* 1) ARUP Institute for Clinical and Experimental Pathology and ARUP Laboratories; 2) Department of Pathology, University of Utah, Salt Lake City, UT.

Biotinidase deficiency (BTD) is an autosomal recessive disorder in which the body cannot process or recycle the vitamin biotin. Over 100 mutations have been identified in the BTD gene that causes biotinidase deficiency. One of every 60,000 newborns is affected with complete or partial BTD. Left untreated, complete BTD causes seizures, hypotonia, developmental delay, rashes, alopecia, hearing loss, or ataxia. Untreated partial BTD, causes any of these symptoms but they may be mild or only exhibited when the individual is stressed or suffering from illness. Current diagnosis of BTD depends on clinical presentations and biotinidase enzyme activity. We developed a sequencing analysis to detect the mutations in the coding region and exon/intron boundaries of the BTD gene. DNA was extracted from 42 serum samples from individuals previously shown to have low biotinidase enzyme activity levels, and 9 whole blood samples from normal individuals. PCR of the BTD gene coding region was done followed by bidirectional DNA sequencing. Data analysis was performed using Mutation Surveyor software. Seventeen of 51 samples failed to amplify in one or more amplicon. Of the remaining 34, 12 samples contained 2 known mutations, 2 samples contained 1 known plus 1 unknown mutation, 1 contained a novel point mutation and a novel 1 bp deletion, while 19 samples contained no mutation. Five new mutations were detected in these samples. In all, 25 of 34 results (74%) correlated with the genotype expected based on enzyme activity. The other 9 samples may have had low enzyme activity levels caused by improper sample handling. Sequencing of the coding regions and intronic boundaries of the BTD gene can be used as a 2nd tier test after an abnormal newborn screen to detect known variations and help define previously unknown BTD variations. A larger sample population using paired controls is needed to verify proper sample handling conditions. A combination of genotype and enzyme activity using paired controls will provide an accurate diagnosis of biotinidase deficiency.

Discordant expression of *PANK2* and *miR-103-2*: a microRNA within its pantothenate kinase-associated neurodegeneration gene host. *B. Polster, M. Yoon, S. Westaway, S. Hayflick* Molecular and Medical Genetics, Oregon Health and Science University, Portland, OR.

Pantothenate kinase-associated neurodegeneration (PKAN) is an autosomal recessive neurodegenerative disorder with dystonia, pigmentary retinopathy, and iron accumulation in brain. The causative gene, *PANK2,* is a member of a family of homologous genes encoding pantothenate kinase. Interestingly, these genes host a family of microRNAs within their introns 5. *PANK1* contains *miR-107*, while *PANK2* and *PANK3* contain *miR-103-2* and *miR-103-1*, respectively. The mature microRNA sequences of *miR-103-1* and *miR-103-2* are identical, while mature *miR-107* differs by a single nucleotide, suggesting shared mRNA targets. The location of *miR-103/107* within the *PANK* genes has high evolutionary conservation. However, the significance of *miR-103/107* preservation within pantothenate kinase transcripts remains unclear.

We hypothesized that *miR-103-2* and *PANK2* would have identical expression patterns and overlapping function, which might give insight into PKAN pathogenesis. Surprisingly, we have demonstrated that *miR-103/107* expression does not correlate with each host pantothenate kinase gene's expression in various mouse tissues using qRT-PCR. In addition, expression of *miR-103-2* precursor and mature *miR-103* is discordant, as demonstrated by northern blot analysis. This difference may be due to post-transcriptional regulation of the microRNA, resulting in distinct *miR-103-2* and *PANK2* expression patterns despite their genomic colocalization. These findings shed light on intronic microRNA processing and highlight the complexity of *PANK2* structure and its potential role in PKAN pathogenesis.

Imputation of allele frequencies at untyped SNPs from summary (or pooled) genotype data. *X. Wen*[1], *M. Stephens*[1,2] 1) Department of Statistics, Univerisity of Chicago, Chicago, IL; 2) Department of Human Genetics, University of Chicago, Chicago, IL.

Recent studies have shown that accurate genotype imputation can greatly increase the power of detecting genetic variation in genome-wide association study. However, most existing imputation methods require individual-level genotype data. In practice, for meta-analysis in genome-wide association studies, there are situations that only summary-level statistics are available; Also, as an alternative to high-throughput genotyping technology, DNA pooling is considered to be more economic and efficient, but provide only summary-level data and not individual genotype data. In these cases, it is desirable to have an imputation method utilizing only summary-level information. Here, we introduce a new statistical method that can efficiently infer the frequency of untyped SNPs using only population panel data (e.g. Hapmap data) and allele frequencies from typed SNPs in the study sample. This approach is a natural extension of Hidden Markov Models (HMM) from Li and Stephens (2003) and Scheet and Stephens (2005), both of which have been successfully employed for genotyped imputation for individual-level data. We also explicilty consider the uncertainties of observed allele frequencies, which is an attractive feature for dealing with data from DNA pooling experiment. The result from real data simulation study and real data application shows that the proposed method yields accurate imputation result and reduces error rates of allele frequency estimates.

Identification of POLG2 mutations in the patients with mitochondrial disorders. *FY. Li, LY. Tang, S. Zhang, ES. Schmitt, LJ. Wong* Molecular and Human Genetics, Balor College of Medicine, Houston, TX.

DNA polymerase gamma is the only DNA polymerase in mammalian mitochondria. It plays a critical role in the biogenesis of mtDNA. The holoenzyme of DNA polymerase gamma consists of one 140-kD catalytic subunit, POLG, and two 55-kD accessory subunits, POLG2. Mutations in the POLG gene have been associated with a broad spectrum of heterogeneous disorders including PEO, Alpers syndrome, MNGIE, SANDO, parkinsonism and premature menopause. To date, over 100 mutations in the POLG gene have been recorded while there is only 1 heterozygous missense mutation in the POLG2 gene reported in 1 PEO patient. We sequenced the entire coding exons of the POLG2 gene in 150 samples, which were negative for POLG mutations (113) or had only one POLG mutation identified (37). This study identified 5 heterozygous missense variants and 1 frame-shift mutation (p.D386E, p.L153V, p.S423Y, p.P205R, p.R369G and c.1423_1424delTT (p.L475DfsX2)) in 7 patients. All missense variants change evolutionarily conserved amino acids. The SIFT and PolyPhen algorithms predict three variants (p.S423Y, p.P205R and p.R369G) to be deleterious and two variants (p.D386E and p.L153V) to be benign. Patients with the POLG2 mutations/variants were all negative for POLG mutations, suggesting that a synergistic effect of di-genic heterozygous mutations is probably not the mechanism of the disease in these patients. Only one patient had clinical symptoms consistent with autosomal dominant (ad) PEO. These patients with POLG2 mutations/variants presented with multisystemic symptoms involving in at least 2 systems (CNS, neuromuscular, visceral or other systems). CNS symptoms such as hypotonia, development delay, seizures or abnormal MRI (6/7), and the visceral symptoms such as gastrointestinal reflux, delayed gastric emptying, constipation or hepatic failure are prominent (7/7). Two patients (2/7) presented with severe multisystemic clinical symptoms at 2 years of age without PEO, a clinical course very different from the adPEO. Longley et al (2006) identified only 1 heterozygous POLG2 mutation by screening 101 patients with PEO. Thus, PEO may not be the major clinical symptom associated with POLG2 mutations.

Comparison of clinical features between *SIL1* mutation -positive and -negative patients in Marinesco-Sjögren syndrome. *A. K. Anttonen[1], I. Mahjneh[2], A. E. Lehesjoki[1]* 1) Folkhälsan Institute of Genetics, Department of Medical Genetics, and Neuroscience Center, University of Helsinki, Helsinki, Finland; 2) Department of Neurology, Pietarsaari Hospital, Pietarsaari, and Department of Neurology, University of Oulu, Oulu, Finland.

Marinesco-Sjögren syndrome (MSS, MIM 248800) is an autosomal recessive disorder affecting various tissues and organs. The main clinical findings are cerebellar ataxia with cerebellar atrophy, early-onset cataracts, psychomotor delay, progressive muscular weakness, and muscle atrophy. In addition, hypergonadotropic hypogonadism, skeletal abnormalities, short stature, strabismus, hypotonia, dysarthria, and nystagmus are frequent findings. Recently, mutations in the *SIL1* gene, which encodes an endoplasmic reticulum resident cochaperone, were identified as a major cause of MSS. However, some patients with typical MSS do not have identifiable mutations in *SIL1*, implying genetic heterogeneity. We have previously excluded functional candidate genes *HSPA5*, *HYOU1*, and *AARS* as genes underlying MSS. Here, we report the results of an ongoing mutation analysis in yet another candidate gene, *CTDP1*, which has been implicated in the congenital cataracts facial dysmorphism neuropathy (CCFDN) syndrome resembling MSS. Moreover, we review the clinical features of the patients with and without *SIL1* mutations in more detail. Our preliminary data imply that patients with *SIL1* mutations form a uniform group whereas *SIL1* mutation-negative patients have more heterogeneous clinical features.

The Integration Of Transcriptional Profiling And Functional Genetics Reveals RBM24 To Be A Novel Candidate For Cardiac Defects. *R. Miller[1], A. Bertoli-Avella[3], B. de Graaf[3], M. Wessels[3], J. Gearhart[4], D. McGaughey[1,4], A. McCallion[1,2]* 1) McKusick-Nathans Inst Gen Med, Johns Hopkins Univ, Baltimore, MD; 2) Department of Molecular and Comparative Pathobiology, Johns Hopkins Univ, Baltimore, MD; 3) Departrments of Clinical Genetics, Erasmus Medical Centre, 3016 AH Rotterdam, The Netherlands; 4) Institute for Cell Engineering, Johns Hopkins Univ, Baltimore, MD.

Cardiogenesis is the result of a complex and coordinated series of events. Perturbations of these processes can lead to congenital heart defects, the most prevalent of all birth defects (7/1000 live births). To better understand cardiac development, we determined the transcriptional profile of mouse embryonic stem cells (mESCs) as they differentiate along a cardiac lineage. By comparing the profile of differentiating cardiomyocytes (DCMs) with time-matched non-DCMs and undifferentiated mESCs we have identified genes whose expression is enriched in DCMs. We have determined the temporal/spatial expression of 31/133 (23%) novel candidates identified in this screen by RNA in situ hybridization at key points during cardiogenesis (E7.5, E8.5, E9.5). All candidates evaluated were expressed in key cardiac structures, with 9/31 detected as early as the formation of the cardiac crescent. One gene identified in this study, Rbm24, was selected for functional evaluation in zebrafish. The zebrafish homolog, zgc:136803 (rbm24, 92.6% identity by AA comparison) recapitulates the cardiac and somitic expression observed in mouse. Injection with translation blocking morpholinos against rbm24 resulted in cardiac looping defects and cardiac edema as well as defects in somite formation. Co-injection of the full-length transcript with the morpholino resulted in phenotype rescue of 62%of injected embryos. Importantly, the human ortholog (RBM24) lies on chromosome 6p22.3, within an interval linked to the segregation of congenital cardiac abnormalities (6p24.3-21.2) in a three-generation European family (nine affecteds). We will discuss this work, our ongoing mutation detection efforts at this locus, and associated functional evaluation of RBM24 coding and non-coding sequences.

A GWAS study of 17,500 individuals and replication in a further 19,000 individuals reveals new loci associated with plasma lipoprotein levels in humans. *D. M. Waterworth[1], S. Debenham[2,3], K. Song[1], E. Wheeler[9], N. Lim[1], J. H. Zhao[3], X. Yuan[1], T. Johnson[4], J. Chambers[8], P. Vollenweider[5], J. Kooner[8], K. Papadakis[6], D. Strachan[6], M. Boehnke[7], P. Deloukas[9], G. Waeber[5], L. Cardon[1], N. Wareham[3], V. E. Mooser[1], M. Sandhu[2,3], GEMS and Sardinia Investigators*
1) Genetics, GlaxoSmithKline, King of Prussia, PA; 2) Department of Public Health and Primary Care, University of Cambridge,Cambridge, UK; 3) MRC Epidemiology Unit, Institute of Metabolic Science, Cambridge, UK; 4) Department of Medical Genetics, University of Lausanne and CHUV, Lausanne, Switzerland; 5) Dept Internal Medicine, CHUV, Lausanne, Switzerland; 6) Division of Community Health Sciences, St Georges, London, UK; 7) Center for Statistical Genetics and Department of Biostatistics, Ann Arbor, Michigan; 8) Department of Epidemiology and Public Health, Imperial College London, UK; 9) Wellcome Trust Sanger Institute, UK.

Plasma levels of lipoproteins are important risk factors for cardiovascular disease. In order to find novel loci associated with circulating HDL-C, LDL-C and triglyceride levels, we conducted a meta-analysis of GWAS summary data from up to eight studies, comprising around 17,500 individuals and approximately 2.2 million genotyped and imputed SNPs. Using a screening threshold of $p< 1 \times 10^{-5}$, we identified 11, 14 and 16 putative novel loci associated with HDL-C, LDL-C and triglycerides, respectively. Of the novel loci detected, a locus on chromosome 9 reached genome-wide statistical association, specifically for LDL-C ($p = 1.2 \times 10^{-10}$). Replication analysis of these loci in 19,000 individuals is now underway. So far, we have found evidence for replication of a novel locus on chromosome 11 that is associated with both HDL-C and triglycerides ($p = 1.5 \times 10^{-9}$ and 2.8×10^{-8} in the combined analysis) and another locus on chromosome 9 ($p = 2.2 \times 10^{-8}$) for HDL-C. We are also examining associations between these novel genetic variants and risk of coronary artery disease (CAD) in a consortium of case-control studies linking the risk factor trait with its clinical phenotype.

Interacting variants in the nicotinic receptor subunit genes influence nicotine dependence risk. *N. L. Saccone[1], S. F. Saccone[2], A. L. Hinrichs[2], J. A. Stitzel[3], W. Duan[1], M. L. Pergadia[2], A. Agrawal[2], N. Breslau[4], R. A. Grucza[2], D. Hatsukami[5], E. O. Johnson[6], P. A. F. Madden[2], G. E. Swan[7], J. C. Wang[2], A. M. Goate[1,2], J. P. Rice[1,2], L. J. Bierut[2]* 1) Dept of Genetics, Division of Human Genetics, Washington University School of Medicine, St Louis, MO; 2) Dept of Psychiatry, Washington University School of Medicine, St. Louis, MO; 3) Dept of Integrative Physiology, Institute for Behavioral Genetics, University of Colorado, Boulder, CO; 4) Dept of Epidemiology, Michigan State University, East Lansing, MI; 5) Dept of Psychiatry, University of Minnesota, Minneapolis, MN; 6) Research Triangle Institute International, Research Triangle Park, NC; 7) Center for Health Sciences, Stanford Research Institute International, Menlo Park, CA.

Recent research has underscored the importance of specific cholinergic nicotinic receptor subunit (*CHRN*) genes in risk for nicotine dependence, smoking, and lung cancer. Our study has surveyed genetic variation across the complete family of 16 *CHRN* genes, which encode the nicotinic acetylcholine receptor (nAChR) subunits, in the NICSNP consortium sample of 1050 nicotine dependent cases and 879 non-dependent controls. These subjects of European descent were recruited from the United States and Australia and assessed using Fagerström criteria. Our previously reported association between nicotine dependence and rs16969968, a non-synonymous SNP in *CHRNA5*, has since been replicated in multiple independent samples via analyses of the same SNP or strong linkage disequilibrium proxies. To further investigate the role of this confirmed locus together with other members of this important gene family, we genotyped additional SNPs in the *CHRN* genes and tested biologically motivated interaction hypotheses. Certain nAChR subunits are known to combine in receptors with the alpha5 subunit, and SNPs across these *CHRN* genes were tested for statistical interactions. Results demonstrate that the non-synonymous SNP rs16969968 in *CHRNA5* interacts with multiple SNPs in *CHRNA4* to further influence risk for nicotine dependence. These findings may inform future efforts to improve smoking cessation treatment.

Three dimensional evaluation of facial structures in autism spectrum disorders. *K. Angkustsiri[1,2], K. Camilleri[2], C. Nordahl[2], C. Li[3], T. Simon[2], R. Hansen[1,2], D. Amaral[2], S. Boyadjiev Boyd[2,4]* 1) Dept Pediatrics, Section of Developmental-Behavioral Pediatrics, University of California, Davis, Sacramento, CA; 2) Medical Investigation of Neurodevelopmental Desorders (M.I.N.D.) Institute, Sacramento, CA; 3) Dept Biostatistics, UC Davis, Sacramento, CA; 4) Dept Pediatrics, Section of Genetics, University of California, Davis, Sacramento, CA.

Background: There is clinical heterogeneity among the autism spectrum disorders (ASD). Identification of a physical phenotype is difficult due to the subjective nature of conventional dysmorphology assessments. We apply indirect anthropometry using 3dMD technology to objectively compare facial features between children with ASD and typical (TYP) development and investigate the association with total cerebral volume (TCV). Methods: As part of a longitudinal study on biomedical and behavioral phenotyping of ASD, 3D images and MRI scans were obtained on 17 Caucasian male (12 ASD, 5 TYP) participants between the ages or 36-47 months. Twelve facial landmarks were placed on the 3D images, generating 14 measurements and 3 indices, which were compared between the groups using two-sided Wilcoxon-Mann-Whitney tests. Spearmans rank-order correlation was used to investigate the association between TCV and each of the 14 measurements and 3 indices. Results: Preliminary analyses revealed no statistically significant differences in any of the measurements or indices between groups. There was an inverse relationship between nasal width and TCV, coefficient=-0.58, 95% CI (-1;0.15). Conclusions: Although our sample size is small, the suggested inverse relationship between nasal width and TCV may be a useful biomarker for children with abnormal cerebral volumes for whom brain imaging should be pursued. We will continue to obtain 3D and MRI data on additional participants and will pursue further analysis, including measures of disproportion using a craniofacial variability index (CVI) and symmetry.

Altered Expression of microRNAs in Post-mortem Brain Samples from Individuals with Major Psychosis. *M. P. Moreau[1], S. E. Bruse[2], R. David-Rus[1], S. Buyske[3,1], L. M. Brzustowicz[1]* 1) Dept Genetics, Rutgers Univ, Piscataway, NJ; 2) Mirus Bio Corp, Madison, WI; 3) Dept Statistics, Rutgers Univ, Piscataway, NJ.

Schizophrenia is a debilitating psychiatric disorder characterized by severely disturbed thought, perception, emotion, and behavior. The association of psychotic features with severe mood disorders, combined with recent genetic insights, suggests that schizophrenia and bipolar disorder are etiologically related. The discovery of small, functional non-coding RNAs has unveiled a layer of gene expression regulation that was completely unknown only a decade ago. MicroRNAs comprise a growing class of endogenous molecules that regulate gene expression post-transcriptionally. Enrichment of predicted miRNA target sites within brain-expressed mRNAs, as well as emerging functional evidence, suggest that miRNAs serve important neurobiological roles. We have performed comprehensive expression analysis of 435 miRNAs and 18 small nucleolar RNAs using TaqMan real-time PCR methodology. Expression signatures were obtained from post-mortem brain samples originating from matched sets of individuals with schizophrenia, individuals with bipolar disorder, and psychiatrically healthy controls (n = 35 each group). Sample covariates pertaining to demographic variables, sample handling, and substance exposure history were assessed using Bayesian model averaging (BMA). BMA revealed that sample storage time and antipsychotic treatment history were most likely to influence miRNA expression signatures. After standardizing miRNA expression values with respect to all sample covariates, 30 miRNAs were found to be significantly under-expressed in individuals with bipolar disorder relative to controls at a false discovery rate less than 10%. While these same miRNAs displayed a trend towards under-expression in individuals with schizophrenia, only one was found to be significantly misexpressed.

Worldwide variation in the serotonin transporter gene SLC6A4. *J. D. Murdoch, W. C. Speed, N. Mukherjee, K. K. Kidd* Department of Genetics, Yale University School of Medicine.

The serotonin transporter gene SLC6A4 displays significant genetic diversity within and between human populations; much of the literature has focused on variation within the promoter VNTR (or HTTLPR) and the intron 2 VNTR. Both have been implicated in a variety of neuropsychiatric conditions and behaviors, though results are often inconsistent among studies. The common promoter VNTR alleles are often described as 16-repeat long and 14-repeat short alleles. However, this ignores additional variation in number of repeat units and repeat unit structure; variation of both types has been described previously. Through resequencing to date of over 100 individuals from our global population samples, we replicated previously described variation and identified several novel rare alleles, confirming that actual allele types are far more numerous than reflected in much of the clinical literature. Most of these alleles are at very low frequency, but display distinct geographic distributions; e.g. a particular 20-repeat allele was observed only in East Asian and Pacific Island populations. Trends in allele frequencies across the globe showed the 16-repeat class to be the most frequent size class in Africa and somewhat less frequent in Europe, while the 14-repeat class is the most frequent in East Asia and the Americas. Resequencing of individuals from several other primate species showed that the 16-repeat human class of alleles is more homologous to the gorilla 16-repeat allele than to any other non-human primate allele. In contrast, resequencing of the intron 2 VNTR in individuals from around the world so far shows no novel repeat number variants or structural variants, and the 12-repeat allele was the most frequent allele worldwide. However, as with the promoter VNTR, rarer alleles did show discrete geographic distributions, e.g. the 9-repeat allele was observed only in European populations. Our findings demonstrate that precise allele characterization and awareness of potential population stratification are needed when performing association studies on either VNTR in SLC6A4, and warrant further investigation into the evolutionary histories of the VNTRs.

Variants in four genomic regions are associated with Aspergers Disorder. *D. Salyakina[1], DQ. Ma[1], J. Jaworski[1], I. Konidari[1], S. Slifer[1], R. Henson[1], D. Martinez[1], H. H. Wright[2], R. K. Abramson[2], J. R. Gilbert[1], J. L. Haines[3], M. A. Pericak-Vance[1], M. L. Cuccaro[1]* 1) MIHG, University of Miami, Miller School of Medicine, Miami, FL; 2) School of Medicine, University of South Carolina, Columbia, SC; 3) Center for Human Genetics Research, Vanderbilt University, Nashville, TN.

Background: We present the first genome-wide scan for a homogeneous subset of autism spectrum disorder (ASD) families defined by individuals who meet research diagnostic criteria for Aspergers Disorder (AS). AS is distinguished from other ASDs by the absence of delays in language and cognitive development. **Method:** Association testing was performed in 103 families genotyped on Illumina 1M beadchip. 804349 SNPs passed quality control (genotyping rate > 0.99, MAF > 0.05, HWE (p-value) > 10^{-5}). **Results:** We identified associations between AS and loci on chromosome (chr) 2p21. Thirty-one of sixty (> 50%) tested SNPs in this region (~150 kb) show $p < 0.05$ to $2.67*10^{-5}$, and lie within the SOCS5 gene that has not been previously tied to ASD. SOCS5 belongs to the suppressor of cytokine signaling (SOCS) family, also known as STAT-induced STAT inhibitor (SSI) protein family. Similar over-representation of significant p-values suggests loci on 20q13.22 (56227710-56385825 bp) and 22q13.33 (48955865-48995704 bp). On chr 20, 45 of 60 SNPs have p-values in the range 0.05 - $8.23*10^{-6}$ and on chr 22, 10 of 18 SNPs show p-values in the range 0.05 - $4.17*10^{-5}$. Candidate genes include PPP4R1L, RAB22A (20q13.22); and PANX2, TRABD and SELO (22q13.33). Chr 3q26.1 also contains a small cluster of SNPs with $p < 10^{-4}$ that lie within a hypothetical gene. **Conclusions:** Our study highlights 4 candidate regions for AS on chr 2, 3, 20 and 22. Although none of these results meets genome-wide significance criteria, this unique set of regions suggests that it is important to collect the full spectrum of ASD to define homogeneous subsets of families. This approach ultimately increases power to detect genetic effects in complex diseases such as autism.

Genetic Variation in an Individual Human Exome. *P. C. Ng, S. Levy, J. Huang, T. B. Stockwell, B. P. Walenz, K. Li, N. Axelrod, D. A. Busam, R. L. Strausberg, J. C. Venter* Genomic Medicine, J Craig Venter Inst, Rockville, MD.

Characterizing the functional variation in an individual is an important step towards the era of personalized medicine. In 2007, we published the genome sequence of J. Craig Venter. Here, we analyze the genetic variation of J. Craig Venters exome, focusing on variation in the coding portion of genes which is thought to contribute significantly to a persons physical make-up. We survey ~12,500 nonsilent coding variants and by applying multiple bioinformatic approaches, we reduce the number of potential phenotypic variants by ~90%. The majority of coding variants in this individual are common and appear to be functionally neutral. Our results also indicate that some variants can be used to improve the current NCBI human reference genome. Our analysis provides a snapshot of the current state of personalized genomics. As sequencing of individual genomes becomes more prevalent, the bioinformatic approaches we present in this study can be used as a paradigm to pursue the study of protein-coding variants for the genomes of many individuals.

Genome-wide profiling for determination of microRNAs involved in brain-related endophenotypes. *M. Carless[1], T. Dyer[1], J. Curran[1], R. Duggirala[1], D. Glahn[2], J. Blangero[1]* 1) Dept Genetics, SFBR, San Antonio, TX; 2) University of Texas Health Science Center at San Antonio, San Antonio, TX.

The genetic determinants of complex brain-related disorders, including schizophrenia, mood disorders, autism and dementia are currently poorly understood and as such, treatment of these debilitating illnesses remains inadequate. Much of the genetic complexity associated with psychiatric disease is related to the phenotypic variation accompanying diagnosis. By focusing on neuroanatomic and neurocognitive endophenotypes as optimal indices of disease risk rather than discrete disease status, the power of a study to identify novel genes can be dramatically increased. We are currently performing genome-wide microRNA expression profiling in a population of 1,000 Mexican Americans across approximately 40 families using the Illumina MicroRNA Expression Profiling Assay. We have extensive brain-related phenotypic data for this population and preliminary analyses have identified a number of microRNA associations with various phenotypes. Of 520 microRNAs that were significantly detected, 304 were identified as heritable using an FDR of 0.05, with a mean heritability of 0.585. We identified 10 microRNA transcripts that exhibited significant associations with total brain white matter volume at an FDR of 0.05. The most strongly correlated of these, *miR-545* ($p = 0.0001$), was also correlated with total brain volume ($p = 0.0018$), gray matter volume ($p = 0.0025$) and a measure of episodic memory ($p = 0.003$). A number of other microRNAs were also found to be strongly correlated with the Eysenck Personality Questionnaire (*miR-100*), working memory (*miR-100*, *miR-30a-3p*), various measures of IQ (*miR-135b*) and the Beck Depression Inventory (*miR-181b*). Interestingly, *miR-181b* has also been implicated in schizophrenia by a small association study. These results, although preliminary, provide strong evidence that microRNA expression levels are associated with a wide variety of neuroanatomic and neurocognitive phenotypes and are therefore likely to be involved in various psychiatric disorders.

Gene Expression Profiling in Aging and Exceptional Longevity. *G. Atzmon[1], T. Budagov[1], R. F. Thompson[1], N. Barzilai[1], A. R. Shuldiner[2]* 1) Dept Med, AECOM, Bronx, NY; 2) Division of Endocrinology, Diabetes and Nutrition, University of Maryland School of Medicine.

The aging phenotype is due to the complex interaction of genetic and environmental factors. Changes in gene expression may be one of the central mechanisms by which aging predisposes to many age-related diseases, and may therefore play a role in lifespan and incidence of age-related degenerative diseases. We systematically assessed changes in gene expression with age by studying global patterns in lymphoblastoid cell lines from 12 male subjects of Ashkenazi Jewish descent (5 probands (i.e. centenarians), and 7 young unrelated controls). We measured gene expression levels with the Affymetrix GeneChip Exon array, representing more than 1.4 million sites across the human genome. Approximately 10% of the analyzed probes demonstrated significant differences between centenarians and controls, 61 of which passed the multiple comparison adjustment (accounting for false positive error). Centenarians exhibited lower expression in 45% of these significant loci compared to the control group. As expected, the number of genes whose expression was altered with age varied according to the length of the individual chromosomes. However, the highest ratio (number of total altered probes/number of total genes per chromosome) of significant altered gene expression was observed on chromosome 2 (average of 2 probes per gene) with more than 30 analyzed probes for the Titin gene (a key component in striated muscles). All 30 (distinct probes representing most of the gene) Titin probes demonstrated significant over expression in centenarians. This is especially interesting as nfi-1 mutant worms that are short lived demonstrate a decrease in Titin gene expression. In summary, our preliminary studies suggest differences in expression of a large number of genes in lymphoblastoid cell lines from centenarians compared to controls. Additional studies of expression patterns may elucidate novel genes and pathways involved in cellular and organismal aging.

Linkage mapping of CVD risk traits in the isolated Norfolk Island population. *C. Bellis[1,2], H. Cox[2], T. D. Dyer[1], K. N. Begley[3], S. Quinlan[2], R. A. Lea[2,4], S. Heath[5], J. Blangero[1], L. R. Griffiths[2]* 1) Southwest Foundation for Biomedical Research, San Antonio, TX; 2) Genomics Research Centre, Griffith Institute for Health and Medical Research, Griffith University, Gold Coast, Australia; 3) Division of Information Services, Griffith University, Gold Coast, Australia; 4) Institute of Environmental Science and Research Ltd, School of Biological Sciences, Victoria University of Wellington, New Zealand; 5) Centre National de Génotypage, 2 Rue Gaston Cremieux, Evry, France.

To understand the underlying genetic architecture of cardiovascular disease (CVD) risk traits, we undertook a genome-wide linkage scan to identify CVD quantitative trait loci (QTLs) in 600 individuals from the Norfolk Island population. The central aim of this research focused on the utilization of a genetically and geographically isolated population of individuals from Norfolk Island for the purposes of variance component linkage analysis to identify QTL involved in CVD risk traits. Substantial evidence supports the involvement of traits such as systolic and diastolic blood pressures (SBP and DBP), high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), body mass index (BMI) and triglycerides (TG) as important risk factors for CVD pathogenesis. In addition to the environmental influences of poor diet, reduced physical activity, increasing age, cigarette smoking and alcohol consumption, many studies have illustrated a strong involvement of genetic components in the CVD phenotype through family and twin studies. We undertook a genome scan using 400 markers spaced approximately 10cM in 600 individuals from Norfolk Island. Genotype data was analyzed using the variance components methods of SOLAR. Our results gave a peak LOD score of 2.01 localizing to chromosome 1p36 for systolic blood pressure and replicated some previously implicated loci for other CVD relevant QTL.

Powerful Association Tests for Genome-Wide Association Studies Using Admixed Populations. *C. Li[1], X. Zhu[2], M. Li[3]* 1) Ctr Human Genetics Research, Dept of Biostatistics, Vanderbilt Univ, Nashville, TN; 2) Dept of Epidemiology and Biostatistics, Case Western Reserve Univ, Cleveland, OH; 3) Dept of Biostatistics and Epidemiology, Univ of Pennsylvania, Philadelphia, PA.

In a population that was recently admixed among different ancestral populations, LD may extend over very long distances. Admixture mapping exploits this unique genetic structure to localize genes that underlie ethnic variation in diseases or traits of interest. However, the regions identified by admixture mapping can often be more than several mega-bases. With the availability of dense markers in genome-wide association (GWA) studies, it is urgent to develop association tests for admixed populations that improve both resolution and power over existing approaches. We developed two novel association tests that simultaneously analyze ancestral states and marker genotypes. The first test is designed for analysis of unrelated cases and controls in GWA studies, and the second test is a case-only test for fine mapping regions identified through traditional admixture mapping. These tests can be extended to joint analysis of multiple markers. Both tests focus on the correlation between ancestry state and genotype score. By bringing these two pieces of information together, our tests offer higher resolution and power than the traditional admixture mapping methods. For example, suppose a disease variant follows a multiplicative model with genotypic relative risk 2.0, and its frequencies are 0.05 and 0.30 in two ancestral populations which were admixed at proportions 80% and 20%, respectively. In this scenario, Montana and Pritchard (2004, AJHG) reported 1,483 cases are needed for admixture mapping to achieve 80% power at genome wide significance level = 0.05. Assuming 5000 SNPs are genotyped in the identified region, with full ancestry information, our method has >99% power to further identify disease-associated SNPs, even at a very stringent significance level of = 0.05/5000.

Effects of glucocerebrosidase (GBA) mutations on proteolytic pathways: The role of autophagy-lysosome and ubiquitin-proteosome systems in GBA-associated parkinsonism. *O. Goker-Alpan, T. Samaddar, BK. Stubblefield, E. Sidransky* MGB/NHGRI, NIH, Bethesda, MD.

Parkinson disease (PD) and other related disorders are caused by deposition of aggregated proteins and/or the failure to clear aggregates leading to neuronal degeneration. The ubiquitin-proteosome system (UPS) and autophagy-lysosome pathway (ALP) are two pathways that remove abnormal proteins. Mutations in genes causing rare familial PD cases implicate UPS dysfunction. Autophagy is the major pathway for the degradation of aggregated proteins, and -synuclein was shown to be cleared by ALP. However, there is not yet a specific gene linking PD to ALP. Recent studies indicate an association between glucocerebrosidase (GC), the lysosomal enzyme deficient in Gaucher disease, and PD and dementia with LBs (DLB). To examine in vivo effects of mutant GC on the pathways implicated in -synuclein clearance, brain samples from subjects with PD or DLB were examined. In samples from subjects with GBA mutations, immunofluorescence studies demonstrated that GC was present in most -synuclein-positive inclusions. In some LBs, GC was present at the core, where aggregates destined for UPS removal often localize. Only 40-60 % of GC-positive LBs were ubiquinated, although all displayed antigenicity against lysosome markers. The effect of glucocerebrosidase on two preoteolytic pathways was examined in a cell-model system over-expressing hA53T-synuclein and wild-type or mutant GBA. UPS function was explored using the small degron CL-1, which demonstrated no influence of either wild-type or mutant GBA on proteosome. The evaluation of ALP function by different methods including following the levels and distribution of the autophagic marker LC3, suggested defective autophagy in cell lines transfected with mutant GBA. GBA mutations may contribute to neurodegeneration by interfering with lysosomal clearance of -synuclein, and lead to PD/DLB pathology.

Synergistic effects of methotrexate (MTX) exposure and MYC inhibition (MYCI) on the growth of Burkitt lymphoma. *L. J. Krueger[1, 2], N. H. Rong[1], L. Tao[1, 2], M. S. Leonard[1, 2], S. P. Dunn[3]* 1) Nemours Biomedical Research, A I DuPont Hosp Children, Wilmington, DE 19803; 2) Department of Biology, University of Delaware, Newark, DE 19716; 3) Department of Surgery, A I DuPont Hosp Children, Wilmington, DE 19803.

In 1982, MYC was initially recognized as a key transforming oncogene and now is found in a wide variety of human tumors including BL, breast, prostate and cervical carcinomas, glioblastomas and osteosarcomas. MYC is a conserved nuclear protein that controls cell growth and differentiation. MYC levels are transiently elevated at the transition from G1 to S in normal lymphocytes, but then rapidly decline. Our previous results on the dysregulation of the non-coding miRNAs in Burkitt lymphoma showed that miRNAs let-7a, let-7b and mir-98 were involved in the dysregulation of MYC. We showed that MYC and let-7a formed an autoregulatory loop. In that study, we proposed that miRNAS may serve in therapeutic applications for Burkitt lymphoma and other human tumors overexpressing MYC (Sampson et al. Cancer Res. 2007). Here, we used compound 10058-F4 which belongs to a class of small molecular inhibitors that function by disrupting MYC-MAX heterodimers in characterizing MYC-MAX dysregulation in the presence of MTX. In Wilson, an EBV- and MYC highly overexpressing BL, effective concentrations of MTX were broad (ranging from 0.1-1.0X10-4 M). The responses were both time- and dose-dependent. Because MTX is specific for the G2 phase of the cell cycle, we anticipated a synergistic response with MYC inhibition. Wilson showed a significant synergism reducing the effective dose of 10058-F4 over 100-fold (0.7 uM). The effects showed decreases in cell number below the initial plating concentration, $p<0.001$. Effects of MYCI were subsequently analyzed by real time gene expression studies for MYC and MYC-regulated genes as well as by western blotting. While 3 other BL lines were responsive to each of the agents, a distinct synergy was not found. This underscores the heterogeneity of BL. In summary, we show the utility of synergy with chemotherapeutic agents and MYC-silencing for increasing inhibition and reducing overall toxicities.

Schizophrenia candidate gene selection and gene networks. *Z. Zhao[1,2], J. Sun[1], B. T. Webb[1], A. H. Fanous[1,3], K. S. Kendler[1]* 1) Virginia Institute for Psychiatric and Behavioral Genetics, Virginia Commonwealth Univ, Richmond, VA; 2) Center for the Study of Biological Complexity,Virginia Commonwealth Univ, Richmond, VA; 3) Dept Psychiatry, Georgetown Univ Medical Center, Washington, DC.

Schizophrenia is a complex and debilitating psychiatric disorder with a lifetime prevalence of ~1. The past decade has witnessed hundreds of reports declaring or refuting claims that candidate genes chosen for their location under linkage peaks or because of their known physiological or pharmacological properties associated with schizophrenia. Identification of potential schizophrenia susceptibility genes is expected to accelerate because of many genome-wide association studies (GWAS) of large samples. It now remains a great challenge for an investigator to decide how to select the data from various sources and weigh and rank the information so that the best candidates can be selected for further investigation (e.g. replication). In this study, we collected and complied the mostly available schizophrenia-associated data including several sets of genome-wide linkage scans, more than 2000 association studies, gene expression data, and extensive literature search, and then selected candidate genes and prioritized them for schizophrenia using a model based data integration (MBDI) procedure. Our evaluation using GWAS results, published expert reviews, and meta-analysis results suggested its utility in follow-up association studies and in further bioinformatics analysis. We next selected 161 top-ranking genes for network analysis. We found that 1) contrary to cancer genes, schizophrenia associated genes tend to be nonessential and do not serve as the network super-hubs; and 2) schizophrenia associated genes have an intermediate level of connectivity in the network (medium-hub). Subnetwork analysis also revealed many important features on their gene regulation and protein interactions. In summary, this study represents the first systematic gene ranking and network analysis for schizophrenia and provides many insights on how schizophrenia susceptibility genes interact with each other and with some environmental factors.

Haplotypes in the surfactant protein-B and interleukin-1 genes are associated with need for mechanical ventilation in children with community acquired pneumonia. *M. K. Dahmer, M. Baldwin, N. Halligan, C. Wang, M. W. Quasney* Children's Research Institute and Department of Pediatrics, Medical College of Wisconsin, Milwaukee, WI.

Community acquired pneumonia is one of the most common infections of childhood and accounts for a significant number of admissions to pediatric intensive care units. Previously we have demonstrated that a particular single nucleotide polymorphism (SNP) in the surfactant protein-B gene (SFTPB) is associated with the need for mechanical ventilation (MV) in adults with community acquired pneumonia. In this study we examined whether specific SFTPB or interleukin-1 (IL1B) haplotypes were associated with the need for MV in Caucasian children with pneumonia. Children with pneumonia (n= 177) were genotyped at 4 SNPs in the SFTPB gene (rs2118177, rs1130866, rs2077079, rs3024791) and 5 SNPs in IL1B gene (rs1071676, rs1143634, rs1143633, rs3136558, rs1143629). SNP genotypes did not deviate significantly from Hardy Weinberg equilibrium. Haplotypes were generated using Phase 2.1. The need for MV in children with pneumonia was associated with a single SFTPB haplotype, ACAG (p=0.0095, Fisher exact test), with 23% of children with this haplotype requiring MV. A single IL1B haplotype trended toward significance, CTGCT (p=0.048), with 21% of children with this haplotype requiring MV. When both the SFTPB and IL1B at risk haplotypes were considered together 35% of children with both haplotypes required MV, 18% of children with only the SFTPB at risk haplotype required MV, 11% of the children with only the IL1B at risk haplotype required MV and only 6% of children with neither required MV (p= 0.0009, Cochran Armitage trend test). These results suggest genetic variation in both the SFTPB and IL1B gene may influence the severity of illness in children with pneumonia. Furthermore, consideration of both SFTPB and IL1B haplotypes may be more informative than either alone.

Gene expression profile of surgical specimens obtained from patients with familial and sporadic forms of mesial temporal lobe epilepsy. *C. V. Maurer-Morelli[1], C. S. Rocha[1], R. R. Domingues[1], H. Tedeschi[2], E. De Oliveira[2], F. Cendes[2], I. Lopes-Cendes[1]* 1) Dept Medical Genetics, Faculty of Medical Sciences, University of Campinas - UNICAMP, Campinas, SP, BRAZIL; 2) Dept Neurology, Faculty of Medical Sciences, University of Campinas - UNICAMP, Campinas, SP, BRAZIL.

Rationale: Mesial temporal lobe epilepsy (MTLE) is the most common and severe form of focal epilepsy and it is frequently associated with the pathological finding of hippocampal sclerosis (HS). Patients with MTLE and HS who are pharmacoresistant may undergo unilateral surgical excision of hippocampal formation as an alternative for seizure control. To address questions related to the pathophysiology of HS in the context of MTLE, we aimed to determined gene expression profiles in hippocampal tissue from patients with pharmacoresistant MTLE who underwent surgical treatment. In addition, we want to compare gene expression patterns in familial and sporadic forms of MTLE. Methods: Eight surgical specimens were obtained from patients with MTLE (n=4 familial MTLE and n=4 sporadic MTLE), as well as control tissues (n=3) from autopsy. This study was performed using the Human Genome U133 Plus 2.0 array (Affymetrix). We used 5 g of total RNA in the One-Cycle Target Labeling protocol (Affymetrix). Data was acquired by GeneChip Scanner 3000 (Affymetrix) and analyzed using MAS5 expression measure (Affymetrix). Data analysis was performed using Bioconductor packages (Affy, Limma and Multtest) and Wilcox-Mann-Whitney test was used for statistical analysis. Results: We found 300 genes which were differentially expressed in samples from patients with MTLE as compared to controls (up and down regulated). In addition, we identified a significantly different gene expression profile in samples from patients with familial and sporadic MTLE. Conclusions: This is the first study using microarray in MTLE with HS to explore the differential gene expression between familial and sporadic forms of MTLE. Our results clearly show that, although similar in the clinical, imaging and neuropathologic features, the familial and sporadic forms of MTLE have distinct molecular signatures. Supported by FAPESP and CNPq.

Meta-analyses of genome-wide association studies for 2-hour Glucose and Insulin levels during an Oral Glucose Tolerance Test. *R. Saxena[1,2], M. F. Hivert[2], V. Mayor[3], R. M. Watanabe[4] for MAGIC* 1) Broad, Cambridge, MA; 2) MGH, Boston, MA; 3) CHUV, Lausanne, Switzerland; 4) USC, Los Angeles, CA.

Two-hour post-load glucose measured during the oral glucose tolerance test (OGTT) defines glucose tolerance and correlates with cardiovascular risk, whereas OGTT two-hour insulin may predict type 2 diabetes. Both measures may also reflect insulin resistance. Defining genetic factors influencing these diabetes-related quantitative traits might lead to better understanding of diabetes pathophysiology and cardiovascular complications. We combined results across four genome-wide association studies (GWAS) for 2-hour glucose and three GWAS for 2-hour insulin through a collaborative effort, MAGIC (Meta-Analyses of Glucose and Insulin-related traits Consortium). Component studies included CoLaus, DGI, the Framingham Heart Study (FHS) and FUSION for 2-hour glucose and DGI, FHS and FUSION for 2-hour insulin. Genotypes were generated using the Affymetrix 500k SNP Array (+MIPS 50K chip for FHS) or the Illumina HapMap 317k Array and imputed for ~2.1 million additional HapMap SNPs. An additive genetic model with study-specific covariates was used to test for genetic association. GWAS results across 6,171 individuals for 2-hour glucose and 4,532 individuals for 2-hour insulin were combined by a z-score-based fixed-effects meta-analysis. No association signal achieved genome-wide significance, however a small excess of signals was observed for both phenotypes. Strongest associations for 2-hour glucose include variants in an unannotated region of chr 18q (meta-analysis P=9.2x10^{-7}), at *MAML3*, an activator of NOTCH receptors (P=3.3x10^{-6}), near the EGF-like-domain 11 gene (*EGFL11*;P=4.3x10^{-6}), and near angiotensin II type 1 receptor (*AGTR*; P=4.8x10^{-6}). The strongest associations for 2-hour insulin were at SNPs near the *CREB3L2* transcription factor (P=1.9x10^{-6}), neurotrophic factor *GDNF* (P=3.2x10^{-6}), serine-threonine kinase *PCTK3* (P=5.4x10^{-6}) and the metalloprotease *ADAMTS5* (meta P=5.5x10^{-6}). In conclusion, we identified several promising candidate loci, and ongoing replication in additional samples will establish whether these variants represent novel genes for 2-hour glucose or 2-hour insulin.

Using Genome-wide SNPs to characterize admixture in African Americans and an African Caribbean population. *T. Murray[1], W. H. L. Kao[1], T. Beaty[1], R. Mathias[2], N. Rafaels[1], M. Faruque[3], H. Watson[1], I. Ruczinski[1], G. Dunston[3], K. C. Barnes[1]* 1) Johns Hopkins Bloomberg School of Public Health, Baltimore , MD; 2) National Human Genome Research Institute, Baltimore, MD; 3) National Human Genome Center at Howard University, Washington, DC.

Admixture is a potential source of confounding in genetic association studies, consequently, detecting admixture and estimating its impact is important. Populations of African descent in the U.S. and the Caribbean share similar historical backgrounds but the distributions of African admixture might differ.We selected 416 genome-wide ancestry informative markers (AIMs) to estimate and compare admixture proportions in 906 African Americans (AAs) and 298 Barbadians (ACs) using STRUCTURE. AAs on average were 72.5% African, 19.6% European and 8% Asian. ACs were 77.4% African, 15.9% European and 6.7% Asian. A Wilcoxon rank sum test of differences in distribution of African ancestry between AAs and ACs yielded p-value <10-19. A principal components analysis based on AIMs yielded one significant eigenvector that explained 48.1% of the variation. An ANOVA test of differences along the first eigenvector between the AAs and ACs yielded a p-value of 2.35x10-16. Estimated Fst between these two populations was 0.006 (SE=0.003), indicating that although Barbadians are on average more African than African Americans, the genetic distance is very small. To compare ancestry estimation between random SNPs and AIMs, we estimated admixture in AAs using 996 uncorrelated SNPs randomly sampled from the genome-wide panel of 622,264 autosomal SNPs. A random subset of 50 markers yielded estimated African, European and Asian ancestries of 63.7%, 21.0% and 15.3%, respectively. For a random subset of 400 SNPs these proportions were 74%, 18.2% and 7.8%, and for the full set of 996, they were 77.2%, 18.1% and 4.6%.Correlations in African admixture estimated by random SNPs and the 416 AIMs were 44% for 50 SNPS, 80.5% for 400 SNPs, and 89.9% for 996 SNPs. These results suggest random markers can detect population substructure in African Americans especially when large numbers of random SNPs are used.

The distribution of positive selection on coding and noncoding sequences across the human genome. *G. A. Wray*[1,2], *C. C. Babbitt*[1], *O. Fedrigo*[1,2], *R. Haygood*[2] 1) Institute for Genome Science & Policy, Duke University, Durham, NC; 2) Dept. of Biology, Duke University, Durham, NC.

The relative contribution of mutations in coding and noncoding regions of the genome to adaptive evolution remain unclear. Several recent studies have surveyed the human genome for evidence of positive selection since the *Pan-Homo* common ancestor. Collectively, these studies constitute a trove of information about adaptation during human origins. We conducted a formal meta-analysis to search for correlations across these studies, focusing on functional categories and tissues of expression. Correlations between categories reveal informative patterns of congruence and heterogeneity between coding and noncoding partitions. We find consistent support across studies for extensive adaptation in several functional categories, including T-cell-mediated immunity, anteroposterior patterning, and the insulin/IGF pathway-protein kinase B signaling cascade. We also find strong contrasts between studies that examined coding and noncoding sequences in terms of enrichment for functional categories and tissues of expression. In particular, it appears that adaptation in developmental processes has occurred largely through noncoding changes, whereas in the immune system this has occurred mainly through coding changes. In addition, signals of positive selection for genes expressed in the CNS are predominately noncoding, in the immune system coding, and in testis both. These results highlight strongly contrasting patterns in the genomic distribution of positive selection among coding and noncoding regions during human evolution.

The genetics concept inventory (GenCI): Identifying and addressing student misconceptions. *S. L. Elrod[1], B. P. D. Bartel[1], J. C. Walz[1], K. M. Polacek[1,2]* 1) Biological Sciences/CESaME, California Polytechnic State University, San Luis Obispo, CA; 2) Fielding Graduate University, Santa Barbara, CA.

College students often graduate with the same scientific misconceptions they had when entering high school; e.g., they believe that air has no weight and thus the carbon dioxide in it cannot contribute to the weight of a plant. The identification of misconceptions is an important step in creating effective learning experiences. A genetics concept inventory (GenCI) has been developed to identify and address current student misconceptions in genetics. Concept inventories are research-based assessment instruments designed to reveal student misconceptions. The GenCI has been given to nearly 400 introductory and upper division genetics students at a large public university in pre- and post-test formats. Our results reveal that students: 1) have a superficial understanding of sister and homologous chromosomes, gene function, and the ubiquity of genetic information in different cell types within a single organism; 2) understand the general nature of alleles, but cannot place this understanding in the context of other concepts such as ploidy; and, 3) can readily identify the products of transcription but are less clear regarding the products of translation. Additionally, our results suggest there are at least two kinds of misconceptions among students: naïve conceptions and alternative conceptions. Naïve conceptions may originate from the use of genetic terms that also have common meanings (e.g., sister, daughter), historical terminology that may over-generalize (e.g., chromosome), or figures in textbooks (e.g., chromosomes represented as X-shaped structures); these should be easily rectified with appropriate instruction. Alternative conceptions are mental models that are not aligned with current scientific evidence, are highly resistant to instruction and can interfere with subsequent learning. Concept inventory and student interview data will be presented, along with suggested instructional interventions and next steps in the development the GenCI. The use of the GenCI is predicted to foster improved instructional approaches and student learning.

Positional cloning of a gene for 46,XY DSD implicates a gene in a common signal transduction pathway in human testis determination. *H. Ostrer[1], S. Shajahan[1], C. LeCaignec[2], S. White[3], A. Friedman[1], J. Willan[4], G. Camerino[5], A. Crosby[6], A. Greenfield[4], A. Sinclair[3], A. Pearlman[1]* 1) New York Univ, USA; 2) CHU Nantes, France; 3) Univ Melbourne, Australia; 4) MRC Mammalian Genetics Unit, UK; 5) Universita di Pavia, Italy; 6) Saint Georges Hosp Med Sch, UK.

Previously, we used linkage analysis to map a gene for familial 46,XY disorder of sex development (46,XY DSD) to the long arm of chromosome 5. Subsequently, we applied the chromosome 5 marker linkage analysis to a second family. The combined, multipoint parametric LOD scores from these two pedigrees (6) favors the existence of a sex-determining locus, which, when mutated, results in 46,XY DSD. When this information was applied to the candidate genes in the critical region on chromosome 5, 11 demonstrated expression above background in developing mouse E10.5 and E11.5 Sertoli cells with none showing greater than 2-fold increases or decreases in expression. For two of these genes, encoding a DNA-binding protein and a signal transduction molecule, respectively, we confirmed the cordal expression in mouse embryonic gonads by in situ hybridization. By DNA sequence analysis, both had genetic variants that were in-phase with inheritance of the phenotype and were not described in the SNPdb database; however, in the upstream region of one gene, an AG transition was observed on 16% of control chromosomes, making it an unlikely cause of the DSD phenotype. A variant in the polypyrimidine track of the splice donor site of exon 2 (IVS2-8TA) of the second gene was not present on control chromosomes. As judged by qPCR and cDNA cloning, this variant disrupted the efficiency of splicing from this site, creating a novel in-frame splice donor at the site of the mutation that resulted in the addition of 2 amino acid residues (IQ). Subsequently, mutations in this gene were demonstrated in the second family (G616R) as well as in a sporadic case (L189P) of 46,XY DSD. A mouse knockout model for this gene demonstrated no bias in the sex ratios of the mice nor diminution of fertility in male mice. Thus, mutations in this gene appear to result in 46,XY,DSD on the basis of gain-of-function, rather than loss-of function, mutations.

High density SNP association analysis of potential HDL candidate genes reveals molecular architecture and functionally relevant domains: focus on CETP. *I. M. Stylianou[1], A. Edmondson[1], M. Li[2], S. L. DerOhannessian[1], A. Khera[1], M. L. Wolfe[1], B. J. Keating[1], M. P. Reilly[1], D. J. Rader[1]* 1) ITMAT, University of Pennsylvania, School of Medicine, Philadelphia, PA; 2) Department of Biostatistics & Epidemiology, University of Pennsylvania School of Medicine, Philadelphia, PA.

Several genes are known to be associated with variation in human HDL-C levels, though in many cases the molecular architecture of these associations is not known. Basic research and mouse genetics have identified a large number of new genes that plausibly affect HDL metabolism but have not been associated with HDL-C levels in humans. GWAS has proven a powerful means to identify novel genes linked to complex phenotypes such as HDL-C. However, many genes are poorly represented on current arrays and correcting for a large number of tests can lead to rejecting marginally significant genes that are still important. We used a custom candidate gene array using tag-SNPs to extensively cover a large number of known and possible HDL candidate genes to interrogate a population of cases with extreme high HDL-C (>95th percentile; n=665) compared with low HDL-C (<25th percentile; n=703). The most significantly associated SNPs were in CETP ($p<1x10\ 14$). The array contains 100 CETP SNPs and the data reveals detailed association and linkage disequilibrium (LD) in three discrete blocks. Our data indicates that the association of CETP SNPs to HDL is dependent upon which of the three LD blocks the SNP is in, hence the LD blocks have distinct properties regarding HDL regulation. In addition, the proximal LD block, which contains the most significant SNPs, is composed of two distinct LD sub-blocks which is apparent in the candidate gene array data (but not the HAPMAP). A better definition of the genetic architecture of CETP and its relationship to HDL-C will help resolve the relationship between CETP, HDL, and CAD. High-density candidate gene arrays will permit detailed interrogation and reveal insights of candidate genes that extend findings from genome-wide association studies.

Identifying novel genes in brachydactyly type A-1. *L. Racacho[1,2], A. Byrnes[1,2], S. A. Nikkel[3], J. MacKenzie[4], D. E. Bulman[1,2]* 1) Regenerative Medicine, Ottawa Health Research Institute, Ottawa, ON, Canada; 2) Biochemistry, Microbiology and Immunology, University of Ottawa, ON, Canada; 3) Department of Genetics, Children's Hospital of Eastern Ontario, Ottawa, ON, Canada; 4) Department of Medical Genetics, Kingston General Hospital, Queens University, Kingston, ON, Canada.

Brachydactyly type A-1 (BDA1) is a congenital disorder that affects normal bone development and patterning. Affected individuals have short fingers, broad hands and are generally short in stature. We have previously reported linkage to chromosome 5p in a large family resulting in the assignment of BDA1B as a new locus; OMIM #607004. We further refined the locus to an approximately 3 Mb genomic region. We have also shown that missense mutations in the Indian hedgehog gene (IHH) are known to cause BDA1 in approximately 50% of our families. Currently, all of the BDA1 mutations have been clustered within the IHH N-terminal fragment. Taken together, this data supports a crucial role of IHH in normal bone development and the existence of another locus for BDA1. We hypothesize that the causative genes involved in BDA1 pathogenesis are involved in the IHH signaling pathway and as such, represents novel members of the pathway. Our main objective is to identify these causative genes and to characterize their involvement with the IHH pathway in bone development.

Obstetric and Gynecologic Issues in Patients with Hermansky-Pudlak Syndrome. *M. A. Merideth[1,2], A. M. Garcia[1], J. Salas[1], K. O'Brien[1,2], T. C. Markello[1], W. A. Gahl[1,2]* 1) NHGRI, NIH, Bethesda, MD; 2) Intramural Office of Rare Diseases, NIH, Bethesda, MD.

Hermansky-Pudlak syndrome (HPS) is a panethnic autosomal recessive disorder characterized by oculocutaneous albinism, a bleeding disorder, and, in some patients, granulomatous colitis and/or a fatal pulmonary fibrosis. There are 8 known subtypes of HPS caused by mutations in 8 separate genes involved with intracellular vesicle formation and trafficking. The bleeding disorder in HPS is due to platelet storage pool deficiency. Women with HPS often have problems with heavy menstrual periods and excess bleeding during deliveries, yet very little information has been published on the obstetric and gynecologic issues in HPS. To pursue this, we surveyed 45 patients under an NIH IRB-approved protocol. The patients ranged in age from 13-65 y with a median of 33 y, and were diagnosed with HPS at a median age of 22 y. Forty-one of the 45 patients (91%) report a history of heavy menstrual periods. Thirty-one patients were treated with oral contraceptive pills to manage heavy menses, and 21 of 31 reported improvement. Three of 4 patients had improvement of heavy menses after insertion of a Mirena progesterone IUD. Four patients underwent hysterectomy for heavy menses at a median age of 33.5 y. Twenty-one patients have been pregnant with a median of 2 pregnancies (range 1-5). Only four of 21 patients had problems with bleeding during pregnancy. Bleeding problems were reported in 9 deliveries, requiring platelets, blood transfusion or ddAVP in 4. Prophylactic platelet transfusion or ddAVP was given prior to 12 deliveries, with no subsequent bleeding problems reported. Postpartum hemorrhage occurred in 13 deliveries: treatment by surgery, transfusion of platelets or medication was required in 10. Obstetrician/Gynecologists have an opportunity to assist in the diagnosis of HPS patients, particularly since a majority of patients surveyed were diagnosed many years after the onset of menses. The history of a menstrual bleeding disorder, combined with some degree of hypopigmentation, should prompt investigation into the diagnosis of HPS.

Human Variation Panels in the NIGMS Human Genetic Cell Repository: Availability of genotyping data and preliminary analysis. *D. L. Coppock[1], A. Hebert[2], N. Gerry[1], P. Bender[1], J. Leonard[1]* 1) Coriell Cell Repositories, Coriell Inst Medical Research, Camden, NJ; 2) Boston University, Boston, MA.

The NIGMS Human Genetic Cell Repository has made available four panels of 100 samples from Caucasians, African-Americans, Han People of Los Angeles and the Mexican American Community of Los Angeles, CA. These panels have been available for several years and have been distributed widely to the research community. The ethnicity and familial relationships were self reported. The samples were either unaffected samples from the NIGMS Repository or were collected specifically for these panels (Mexican and Chinese samples from Los Angeles). The 400 samples have been genotyped on the Affymetrix Genome-Wide SNP Array 6.0 and the genotype data are available for downloading from Coriell at http://ccr.coriell.org/Sections/Collections/NIGMS/GenotypeCopyData.aspx?PgId=564&coll=GM. An analysis of the samples by panel using Helix Tree showed the Chinese, Caucasian and African-American samples clustered with little overlap, but some of the Mexican samples overlapped the African American samples. An analysis of the amplified or deleted autosomal regions greater than 500 kb for the 400 samples showed there were 289 copy number variations in 66 autosomal regions. These large copy number variants were found in 210 of the 400 samples and were relatively evenly distributed in the four populations. These regions were found in all autosomes except chromosome 18. The most common variants, occurring at least 10 times, were at chr15q11.2, 8p23.1, 2p11.1-2p11.2, 10q11.22, 14q11-14q12, and 1q21.1. A more detailed analysis of smaller copy number variants in the Human Variation Panels will be shown.

An ENU Screen for Mutations that Disrupt Brain Cortical Development in the Mouse. *R. W. Stottmann[1], J. L. Moran[1], D. Cunningham[2], M. A. Kennedy[2], G. E. Herman[2], D. R. Beier[1]* 1) Brigham and Women's Hospital/Harvard Medical School, Boston, MA; 2) Dept Pediatrics, Nationwide Children's Hosp, Columbus, OH.

The forebrain is the largest portion of the human brain and is responsible for many higher order cognitive functions including reasoning and memory. Remarkably, a large portion of the genetic regulation of forebrain development remains unknown. We have completed an ENU mutagenesis screen in the mouse to identify more of the genes required for forebrain development. We use a traditional breeding strategy to obtain autosomal recessive mutations and observe for phenotypes at E18.5. This stage of development, just before birth, allows the embryo to survive with relatively severe defects in organogenesis which would be lethal in a newborn animal. Affected progeny are used directly for genetic mapping utilizing a 768-marker SNP panel that allows a rapid genome-wide analysis. With this strategy, we are able to localize the mutation to a single linkage group by genotyping 8-10 affected mice. The screen presented here has three components: a traditional phenotypic screen complemented by histological analysis, use of a reporter allele to highlight distinct brain structures, and use of a sensitizing allele to increase the incidence of defects in neural developmental *(Lis1/Pafah1b1)*. In this screen we identified thirteen mutations of interest, eight of which affect CNS development. All of these are mapping as monogenic, recessive traits and we have cloned four mutations and identified the gene mutated in a fifth using whole genome polymorphism analyses, microsatellite mapping and microarray analysis. The most remarkable phenotype uncovered to date shows severe cortical agenesis as well as defects in the appendicular skeleton (smaller long bones). This mutation reveals a requirement for embryonic cholesterol metabolism in brain development and possibly in Sonic hedgehog signaling. Other mutations show significant changes in brain morphology. Some phenotypes are present in grossly normal brains with defects only detectable upon histological analysis or by reporter gene staining. These results demonstrate how ENU mutagenesis can be utilized to query specific developmental processes.

Causal penetrance: definition and relevance to 2-locus heterogeneity. *A. M. Madsen[1], S. E. Hodge[2,3]* 1) Epidemiology; 2) Biostatistics, MSPH, Columbia U; 3) NYSPI, New York, NY.

Background: We introduce the concept of causal penetrance (CausPen) = P[D caused by genotype (gt)|gt] in contrast to the usual statistical penetrance (StatPen) = P[D|gt]. CausPen, akin to attributable risk, pertains to the proportion of disease preventable by eliminating the causal effects of the detrimental variant. This measure may be valuable for risk prediction, data simulations, etc. A model used to predict the risk of disease among carriers of two susceptibility genes that independently affect disease risk is Rischs (1990) two-locus heterogeneity formula: $w_{ij}=x_i+y_j-x_iy_j$ (Eq. 1); w_{ij}=penetrance of genotype G_iH_j; x_i and y_j=penetrance summands for genotypes G_i and H_j, respectively. Objectives: To compare the calculated w_{ij} with the true CausPen of G_iH_j for two different estimates of x_i and y_j: the single-locus StatPen and our estimate of CausPen, the risk difference (RD): RD=P[D|gt]-P[D|no gt]. Methods: We define a causal model of complex genetic disease by combining a sufficient component cause (SCC) model of genetic heterogeneity, Greenlands response types, and a counterfactual definition of causation. Our SCC model assumes no epistasis, no imprinting, and independence between genes and environment. We assign different sets of prevalences of the component causes in the SCC model. For each set, we calculate w_{ij} using StatPen and RD to estimate x_i and y_j. We compare these w_{ij} with the true CausPen of G_iH_j. Results: (1)When the single-locus genotypes are neither necessary nor sufficient, w_{ij} does not equal, and usually overestimates the probability that the two-locus genotype will cause disease using StatPen or RD. The overestimation is proportional to the prevalence of causes of disease in the population that do not include locus G or H. (2)Using RD substantially reduces this overestimation. E.g., when the causal G and H genotypes occur with p=0.1, an additional cause of disease occurs with p=0.1, and the true CausPen= 0.84, the StatPen w_{ij}= 0.96 and the RD w_{ij}=0.85. Conclusion: When the CausPen of two loci is of interest, e.g. in some risk and simulation settings, the RD should be used to estimate the penetrance summands of Eq. 1.

A whole-genome association study of severe malaria. *K. S. Small, MalariaGEN Consortium* Wellcome Trust Centre for Human Genetics, Oxford University, Oxford, United Kingdom.

Malaria kills approximately a million children each year, but a much larger number survive despite repeated infections, and it is poorly understood why some children and not others die of malaria. The MalariaGEN Consortium (partner institutions listed at www.malariagen.net)was established to carry out multicentre studies investigating the genetic basis of resistance to malaria. Here we present the results of two whole-genome association studies of severe malaria; a case-control study of 2,500 children in The Gambia, West Africa, and a family-based association study of 1,500 affected-offspring family trios from The Gambia, Ghana and Malawi.

The Effects of Polymorphisms on CPS1 Activity. *S. Reiss[1], L. Hall[2], A. Putnam[1], G. Cunningham[1], M. Summar, M.D.[1]* 1) Pediatrics, Vanderbilt University, Nashville, TN; 2) Medicine, Vanderbilt Universitiy, Nashville, TN.

The urea cycle is the only metabolic process capable of eliminating the bodys accumulation of ammonia by converting ammonia into its easily excreted form of urea. Carbamyl phosphate synthetase 1 (CPS1) is the primary rate limiting enzyme catalyzing the first committed step of the urea cycle by converting ammonia and bicarbonate into carbamyl phosphate in the presence of ATP and N-acetyl glutamate synthetase (NAGS). Variations in this highly conserved enzyme could have significant clinical effects. We have found that one particular change in the CPSI gene, a threonine to asparagine missense change at amino acid 1405, T1405N (het. .44) is strongly associated with in vivo variations in urea cycle intermediates and the ability to produce nitric oxide. We have incorporated both versions of the T1405N polymorphism and both versions of the T344A (het .45) change subsequently. We performed human cell culture expression of the recombinant versions of this enzyme. We detected a significant difference in the activity levels for the 2 versions of T1405N but not for T344A. We also found that co-factor levels of N-acetylglutamate have an effect on the difference in activity between these two versions of T1405N (the change is in the cofactor binding domain). These findings may provide a biochemical rationale for the clinical associations seen with this polymorphism. We wish to demonstrate the effects on CPS1s activity through comparison of the incorporated polymorphisms.

Accommodating uncertainty in SNP calling in population genetic inference from deep resequencing data. *A. G. Clark[1], K. E. Lohmueller[1], X. Liu[2], T. J. Rea[3], A. R. Templeton[4], J. S. Pankow[5], T. Maxwell[2], E. Boerwinkle[2], D. M. Muzny[6], D. A. Wheeler[6], O. Hall[6], L. Bull[6], R. A. Gibbs[6], C. F. Sing[3]* 1) Dept Molec Biol & Genetics, Cornell Univ, Ithaca, NY; 2) Univ of Texas Health Science Center at Houston; 3) Dept Human Genetics, Univ Michigan; 4) Dept Biology, Washington Univ; 5) Univ. Minnesota; 6) Human Genome Sequencing Center, Baylor College Medicine.

While deep resequencing data provides the opportunity to capture rare variants that would be missed by standard genotyping arrays, it gives rise to challenging problems in population genetic inference in the face of uncertainties in the data. Here we performed PCR-resequencing of the HHEX (7.7 kb) and KCNJ11 (5.4 kb) genes in a sample of 1786 individuals as part of a project to identify variants that are associated with inflated disease risk. Inference of association with rare variants and population genetic analysis of recent demographic history both rely heavily on the rarest segregating alleles, precisely those that are likely to suffer the highest miscalling rate. We identified 85 segregating sites with high confidence in KCNJ11 and 148 segregating sites in HHEX (using Sanger sequencing and SNPdetector), and performed analysis by multiple imputation over the cloud of potential SNPs assessed from the base quality scores. The approach generates 1000 data sets, each representing a sampling of the base calls weighted by their phred quality scores. All population genetic parameters are estimated for each such data set, producing distributions of the parameters that reflect the sequencing uncertainty. Counter to the intuition that synonymous and neutral sites might be saturated with a sample of this size, the observed count of segregating sites, their site frequency spectrum, and the pattern of linkage disequilibrium are consistent with standard models of human demography. The variation is sharply at odds with models that assume equal mutability of all sites and homogeneous recombination. Assessment of association with disease in such deep sequence data will be best done if these inhomogeneities can be accommodated in the model.

Polymorphisms in the Genes of key HPA Axis Molecules are Examined for Association to Cushing's Syndrome.
J. T. Papademetriou, A. Horvath, L. Herishanu, S. Verma, E. Bimpaki, S. Boikos, C. Stratakis Section on Endocrinology and Genetics, National Institutes of Health, Bethesda, MD.

The purpose of this study was to examine whether polymorphic variants of key genes of the hypothalamic-pituitary-adrenal (HPA) axis influence the recovery time of patients following surgical cure of Cushings Syndrome (CS). Patient genotypes were accumulated for twelve polymorphisms: including nine single nucleotide polymorphisms (SNPs) on the corticotropin releasing hormone receptor 1 (CRHR1) gene , one on the pro-opiomelanocortin (POMC) gene, and two SNPs on the glucocorticoid receptor (GC) gene. Clinical data indicative of disease severity (pre-operative Urine Free Cortisol levels (UFC), pre-operative Midnight Cortisol levels (MC)), and return to normal HPA axis functionality following Transphenoidal Surgery (TSS)(post-operative cortisol levels 12 months after TSS (C12post)) were collected and assessed for genotypic differences at each polymorphic locus. Four new polymorphisms were discovered and included in our analysis. One of them (CC/CT), located approximately 4,428bp after the splice site of intron 4 of the CRHR1 gene, was associated significantly with pre-operative MC levels: CC patients had MC levels of 28.4 +/- 14.0 pg/mL, whereas CT patients were 6.75 +/- 4.6 pg/mL (p=0.03998). TT patients were not observed in our cohort. All other polymorphisms did not produce significant differences between genotypic groups. We conclude that severity of CS is significantly affected by the genotype of patients at this CC/CT site. Therefore, this polymorphism may be useful as a diagnostic tool, and its role in CS warrants further investigation. All other polymorphisms studied did not show statistically significant genotypic differences in UFC, MC, or C12post.

Chromosomal identification of multiple HER-2/neu loci in an ovarian cyst. *M. Macera[1], W. Thelmo[2], A. Abdu[2], P. Chandra[3], F. Hristescu[1], A. Babu[1]* 1) Dept of Medicine, Div of Molecular Medicine & Genetics; 2) Dept of Pathology; 3) Dept of Medicine, Wyckoff Heights Medical Ctr, Brooklyn, NY.

A 71 year old female was admitted due to increasing abdominal girth for the last 2 months. Upon examination, no vaginal bleeding was observed. She had no previous operations or disease. A huge ovarian cystic mass filling up the pelvis and involving the omentum, weighing 2150 gms, was surgically removed. The uterus, right ovary and fallopian tube were negative for tumor. Cytogenetic analysis of the cyst, growing aggressively in culture, revealed only abnormal cells with a karyotype of: 56~58,X,-X,+ del(1p32), +2, +der(3)t(3;?)(q11:?), +6, +7, +8, +10, +10, i(13q), t(13;?)(p11;?), der(17), +20, +20, i(21q), -22, +2-4mar. FISH analysis revealed 2 copies of CCND1 and three copies of BCL6, with a signal on the der(3). On the der(17), two signals for D17Z1 were detected towards the distal ends, four HER-2/neu sites were identified along the length of the chromosome. The entire chromosome was positive for wcp 17 except for a small segment, which has not been identified. The der(17) can be described as: pterq11.1q25.3::q25.3q11.2::q11.2q25.3::q25.3q11.1:: ?, or der(17)dic(17)inv dup(q11.2q25.3)dup(q11.2q11.2)t(17:?)(q11.1:?) .ish(wcp17+, cep17x2, HER-2x4). Amplification of HER-2/neu, also found in ovarian cancer, is determined by a ratio of the number of copies of HER-2/neu to 17 centromers. Although HER-2/neu is over represented with additional copies in this case, imniohistochemistry staining on the histological section was negative. Some interphase nucli, with 3 centromere, showed more than 15 HER-2/neu copies. None of these were seen at mitosis. These cells appear to represent a second clone that was not observed in mitosis. Further investigation is underway. This study presents a novel cytogenetic mechanism contributing to multiple copies/amplification of HER-2/neu loci.

Analyses of gene and protein variations of Retinoic Acid Induced 1 (*RAI1*) in Smith-Magenis Syndrome. *T. Vilboux[1], A. C. M. Smith[3], A. Garcia[1], C. Ciccone[1], J. Blancato[2], W. Introne[3], W. A. Gahl[1,3], M. Huizing[1]* 1) NHGRI/MGB, NIH, Bethesda, MD; 2) Georgetown Univ, Washington, DC; 3) Office of Rare Diseases, OD, NIH, Bethesda, MD.

Smith-Magenis syndrome (SMS) is a complex developmental disorder involving variable symptoms such as mental retardation, craniofacial dysmorphia, height-growth delay, infantile hypotonia, brachydactyly, attention deficit, decreased sensitivity to pain, self-injury, maladaptive behaviors and sleep disturbance. Disrupted sleep patterns and behavioral problems characteristic of SMS could be related to an inverted diurnal rhythm of melatonin release. The prevalence of SMS is estimated to be 1/25000 but it is likely underdiagnosed. The syndrome is ascribed to a 2-9Mb interstitial deletion on chromosome 17p11.2. A small number of SMS patients lack any deletion, but carry dominant mutations in *RAI1* (*Retinoic Acid Induced 1*), which resides in the common deletion area. Individuals with mutated *RAI1* have many of the major features of SMS. *RAI1* function is unknown. It is highly conserved through mammalian evolution and appears to be a transcriptional regulator, likely involved in neuronal development. We analyzed *RAI1* and its involvement with the clinical features of SMS. We studied 10 patients with the SMS phenotype but without a common deletion, confirmed by FISH and copy number qPCR. We confirmed presence of two copies of *RAI1* in these patients and sequenced RAI1 exons and intronic boundaries for mutations. Multiple variants were found, including known SNPs, but also 3 unreported variants, 2 are amino-acid changing. We are analyzing the patients RNA, isolated from skin fibroblasts and/or lymphoblast cell lines, for variations in *RAI1* transcription or splicing. Next, we will determine RAI1 protein expression through Western blotting to detect variations in translation. Identifying new mutations and understanding how they affect *RAI1* can help to define the precise cellular function of this protein. Determining how a single mutation in *RAI1* can result in the varied clinical features of this disorder may also assist in understanding the pathways involved in craniofacial development, sleep and behavior.

Rank correlations among results of intra-familial tests of association for quantitative traits with low heritabilities. *H. Sung[1], J. E. Herrera-Galeano[1,2], A. J. M. Sorant[1], R. A. Mathias[1], A. F. Wilson[1]* 1) Genometric section, IDRB, NHGRI, NIH, Baltimore, MD; 2) Dept. of Medicine, Johns Hopkins Medical Institution, Baltimore, MD.

Several different methods are now available for testing for associations between quantitative traits and SNPs in family data. These methods use different kinds of information and have different strengths and weaknesses with respect to their statistical properties. In a study of platelet aggregation, Herrera-Galeano et al. [ASHG, 2007] used several different association methods and found little correlation between results. Computer simulation was used to investigate the lack of agreement among methods. Genetic Analysis Simulation Program (G.A.S.P. v3.3) was used to generate 10,000 samples, each with 200 nuclear families with sibship size three. A quantitative trait was simulated based on a single biallelic locus with equally frequent alleles. The underlying genetic model was additive and heritabilities considered included 0, 0.001, 0.005, 0.01, 0.05 and 0.1. The data availability was modeled as complete or 50% missing. Five tests of association were performed: ASSOC (SAGE), FBAT, GEE (SAS GENMOD), ROMP (Regression on Mid-Parent) and ROOP (Regression on One Parent). Pair-wise Pearson correlations of resulting p-values and pair-wise Spearman correlation using ranks of p-values were calculated. In general, pair-wise Spearman rank correlations have higher correlation than Pearson correlation. For example, with complete data and heritability equal to 0.01, Pearson correlation was as low as 0.03 (FBAT and GEE) but Spearman rank correlations were generally over 0.5. However, in general, different association tests did not agree well even in rank, except for ASSOC and GEE.

Medical and Social Obstacles to the Optimal Intake and Care of Patients with Neurofibromatosis Type 1. *C. M. Legendre, R. Drouin, C. Bouffard* Service of Genetics, Department of Pediatrics, Faculty of Medicine and Health Sciences, Université de Sherbrooke, Sherbrooke (Quebec) Canada.

With a prevalence of 1/3000 and an autosomal dominant mode of transmission in 50% of cases, neurofibromatosis type 1 (NF1) is one of the most common genetic diseases worldwide. Paradoxically and for undetermined reasons, it is still largely unknown to both the general public and health professionals. Thus despite its major impact on physical and psychological health and the importance of early diagnosis, NF1 is rarely diagnosed prior to the onset of complications or before affected individuals have had children. It would therefore appear important to better understand the factors that stand in the way of the optimal intake and care of NF1 patients. Methods: Analysis of the literature on the medical, psychological, ethical, and social aspects of neurofibromatosis; informal interviews: Association de la NF du Québec and NF Canada. Results: Late diagnosis of NF1 is often due to unawareness of the disease, which is indeed still confused with Proteus syndrome. Even when it is diagnosed, the consequences of the disease are often minimized. Thus patients are not consistently referred to a genetics service. When genetic counselling is offered, it is poorly adjusted to differences in age. In addition, the psychosocial consequences of adolescence and the onset of physical deformities, the impact of cultural representations on NF, and the absence of intergenerational dialogue are all, despite their significance, neglected by social scientists; and few studies have taken an interest in questions of reproduction and genetic counselling. Conclusion: This ignorance has serious repercussions on patients intake and care, quality of life, and reproductive decisions, and in particular on the development and physical, intellectual, and psychological health of children with NF1.

Genome-wide association study of plasma polyunsaturated fatty acids in the InCHIANTI study. *T. Tanaka[1,2], J. Shen[3], G. Abecasis[4], A. Kisialiou[2], J. M. Ordovas[3], D. Arnett[5], M. Y. Tsai[6], A. Singleton[7], S. Bandinelli[2], A. Cherubini[8], J. Guralnik[1], L. Ferrucci[1,2]* 1) Clinical Research Branch, NIA, Baltimore, MD, USA; 2) Geriatric Rehabilitation Unit-ASF, Florence, Italy; 3) Laboratory of Nutritional Genomics, USDA-HNRCA at Tufts University, Boston, MA, USA; 4) CSG Dpt. of Biostatistics, University of Michigan, Ann Arbor, MI; 5) SPH,Clinical Nutrition Research Center, University of Alabama at Birmingham, AL,USA; 6) Laboratory of Medicine and Pathology, University of Minnesota, MN,USA; 7) Laboratory of Neurogenetics-NIA,MD, USA; 8) University of Perugia, Dept. of Clinical and Experimental Medicine, Italy.

Polyunsaturated fatty acids (PUFA) have multiple physiological roles from substrates for energy production, inflammation modulators, and cell membrane components. High PUFA intake has beneficial effects on cardiovascular morbidity and mortality. To identify genetic contributors to plasma PUFA concentrations, we conducted a genome-wide association study on plasma levels of six omega-3 and omega-6 fatty acids in 1075 subjects of the InCHIANTI study. The first locus with the greatest significance was observed in a region of chromosome 11 with three fatty acid desaturases (FADS). The most significant SNP was rs174537 near FADS1 for arachidonic acid (AA; P=5.95x10-46). Homozygotes for the minor allele had 2.3%; lower AA compared to the homozygotes for the major allele and rs174537 accounted for 18.6%; of the additive variance in AA concentrations. Genome-wide significance was observed for this SNP for eicosadenoic acid (P=6.78 x 10-9) and eicosapentanoic acid (P=1.07 x 10-14). Subject carrying the allele associated with higher AA, EDA, and EPA also had higher LDL cholesterol and total cholesterol levels. The second most significant locus mapped to chromosome 6 encoding an elongase of very long fatty acids 2 (ELOVL2) was associated with eicosapentanoic acid (rs953413; P=1.1 x 10-6). These findings were replicated with an independent sample of 1076 subjects in the GOLDN study where fatty acids were quantified in erythrocyte membrane. Our findings may provide insight into the role of genetics on serum levels of fatty acids.

Modelling mutation and random variation in SNP data as a per-site uncertainty rate. *L. Moutsianas, G. McVean*
Statistics, University of Oxford, Oxford, United Kingdom.

Useful statistical models for capturing genetic variation should explain as much of the observed variability as possible, while retaining their computational tractability. Most widely used models today focus on effects of meiotic crossover and point mutations on genomic data. Many of these use a constant, site-independent mutation rate across extended genetic regions.

Given the confirmed variability of mutation effects across the human genome,we consider a site-dependent mutation rate as a more realistic representation of this biological process. Hence, we introduce a per-site uncertainty rate, not just to replace the site-independent mutation rate, but as an explanatory factor for possible genotyping errors or any other, not explicitly considered, source of genetic or random variation. Based on the imperfect mosaic idea, we have built a Hidden Markov Model which explicitly accounts for crossover and mutation effects. We then employ an Expectation - Maximization algorithm to estimate the per-site uncertainty rates which better explain the observed variation in the sampled data.

Results on data simulated from the coalescent show that our algorithm successfully identifies sites with artificially introduced variation, and assigns high uncertainty rates to them. Results on real data from the HapMap project show a slight improvement in allelic predictions, compared to these obtained using a uniform mutation rate, as well as a more marked one in calibration, especially for the sites with increased uncertainty rates.

Our method can be used to weight marker information from sources of variable quality. Moreover, it should be easily modifiable for use with different types of samples, such as whole sequence data. In addition, it can be extended so as to incorporate other sources of genetic variation, at the expense of increased computational complexity.

Expanded Catalog of eQTLs for Lymphoblastoid Cell Lines. *L. Liang[1], N. Morar[2], M. Moffatt[2], G. M. Lathrop[3], G. R. Abecasis[1], W. O. C. Cookson[2]* 1) Center for Statistical Genetics, Department of Biostatistics, School of public Health, Ann Arbor, Michigan 48109-2029, USA; 2) National Heart and Lung Institute, Imperial College London, London SW3 6LY, UK; 3) Centre National de Genotypage, 91057 Evry Cedex, France.

Gene expression levels can be an important step between DNA variation and phenotypic manifestations. Our initial map of global gene expression based on ~400K SNPs and ~50K transcripts in ~400 sib pairs using Affymetrix arrays helped interpret the results of GWAS for asthma, Crohns disease and Type 1 diabetes, among other traits. Here, we describe an expanded catalogue of expression QTLs (eQTLs), generated by examining (a) additional 550 sib pairs, (b) examining imputed HapMap SNPs and genotyped SNPs, (c) measuring gene expression with Illumina BeadChips. In agreement with our previous results, transcript levels for genes involved in immune response, signal transduction and cell adhesion are highly heritable. We identify significantly associated SNPs for 1,867 genes accounting for 3.5-62.6% of the variance in transcript levels. Analysis of imputed SNPs increases the number of genes mapped in *Cis* by 12.0% to 1,458. To validate the accuracy of imputed genotypes, we compared association results for imputed and genotyped SNPs at 58,268 SNPs (correlation between imputed and true LOD scores was 0.952). Surprisingly, only 17.7% of genes identified using Illumina arrays at $p<10^{-7}$ also show significant association in the Affymetrix data at a similar significance level. The overlap increases to 29.3% when we used a threshold of $p<0.001$ for the Affymetrix data. This expanded eQTL catalog emphasizes the importance of developing even more robust platforms for measuring gene expression and the utility of large datasets. In a preliminary scan of significant eQTL associations that are associated with hit-SNPs from published GWAS (www.genome.gov/gwastudies/), we find ~20 hits from the Affymetrix data and ~35 hits from the Illumina data. There is significant added value from including the Illumina platform and the two platforms provide complimentary information. Both the Illumina and imputation results will be available to the public.

Extending the spectrum of Ellis van Creveld syndrome: a large family with a mild mutation in the EVC gene. *H. Ulucan[1,2], D. Gül[3], J. C. Sapp[1], J. Cockerham[4], J. J. Johnston[1], L. G. Biesecker[1]* 1) Natl Human Genome Res Inst, NIH, Bethesda, MD, USA; 2) Adnan Menderes University Medical Faculty, Department of Medical Genetics, Aydin, Turkey; 3) Gulhane Military Medical Academy, Department of Medical Genetics, Ankara, Turkey; 4) Childrens National Medical Center, Department of Cardiology, Washington, DC, USA.

Ellis-van Creveld (EvC) syndrome is characterized by short limbs, short ribs, postaxial polydactyly, dysplastic nails and teeth and is inherited in an autosomal recessive pattern. We report a family with complex septal cardiac defects, rhizomelic limb shortening, and polydactyly, without the typical lip, dental, and nail abnormalities of EvC. The phenotype was inherited in an autosomal recessive pattern, with one instance of pseudodominant inheritance. Because of the phenotypic overlap with EvC, microsatellite markers were used to test for linkage to the EVC/EVC2 locus. The results did not exclude linkage, so samples were sequenced for mutations. We identified a c.1868TC mutation in EVC, which predicts p.L623P, and was homozygous in affected individuals. We conclude that this EVC mutation is hypomorphic and that such mutations can cause a phenotype of cardiac and limb defects that is less severe than typical EvC. EVC mutation analysis should be considered in patients with cardiac and limb malformations, even if they do not manifest typical EvC syndrome.

Accounting for Disease Model Uncertainty in Mapping Heterogeneous Traits - A Bayesian Model Averaging Approach. *S. Biswas* Department of Biostatistics, School of Public Health, University of North Texas Health Science Center, Fort Worth, TX.

Locus heterogeneity is a ubiquitous feature of complex genetic traits. It refers to the situations when a disease can be caused in different individuals by different genes and/or environmental factors. Recently, a Bayesian approach has been proposed to account for variable rates of heterogeneity across families in a parametric linkage analysis setup (Biswas and Lin, 2006; Journal of the American Statistical Association). As is the case with any parametric approach, its application requires specification of disease model (disease allele frequency and penetrances), which limits its practical utility. To address this limitation, we propose a Bayesian Model Averaging (BMA) approach. We implement BMA in the Bayesian framework proposed by Biswas and Lin (2006) by considering a finite number of disease models and treating model as an unknown parameter with a finite number of categories. In practice, for all our applications we use simple single-locus disease models such as dominant, recessive, and intermediate as various categories for disease model. We first conduct a simulation study wherein true disease models are single-locus models generated under heterogeneity. We show using various simulation scenarios that BMA approach retains at least 80% of the power that is obtained by analyzing under the true disease model. Next we investigate the properties of BMA when the true model is extremely complex consisting of multiple interacting loci. For this, we analyze all 100 replicates of Genetic Analysis Workshop 13 simulated data that were generated to mimic the real data from Framingham Heart Study. Similar results were obtained. This shows that the proposed BMA approach utilizing simple single-locus models for analysis and averaging is effective for mapping heterogeneous traits.

Intracerebroventricular delivery of copper for the treatment of a murine model of Menkes disease. *A. Donsante*[1], *L. Brinster*[2], *S. Lal*[3], *J. A. Centeno*[3], *S. Kaler*[1] 1) UPG, NICHD; 2) DVR, NIH, Bethesda, MD; 3) DPT, AFIP, Washington, DC.

Menkes disease (MD) is a genetic copper deficiency that strikes male infants early in life, resulting in neurodegeneration, seizures, loss of early neurodevelopmental milestones, and premature death. Biochemically, MD is characterized by low concentrations of copper in blood and brain, reflecting impaired transport across the gastrointestinal epithelium and the blood-brain barrier, respectively, due to mutations in an evolutionarily conserved copper transporter, ATP7A. Current treatment is limited to daily subcutaneous copper injections for the first three years of life and is especially effective in patients with mutant alleles that possess some copper transport capacity (N Engl J Med 2008 358:605-614). For patients with complete loss of function mutations, e.g., large deletions, alternative treatment approaches are needed to optimize neurodevelopmental outcomes. We examined the efficacy of direct central nervous system treatment in a murine model of classical Menkes disease, atp7amo-br, caused by a six base deletion in atp7a. We developed a genotyping assay that identified mutant pups at birth prior to clinical manifestions and administered one-time, bilateral intracerebroventricular (ICV) injections of copper chloride (50 ng total) on or before 3 days of age. The dose was extrapolated from the maximum tolerated dose we determined previously in adult rats (Molec Genet Metab 2007 91:30-36). Treatment increased the amount of copper in the brains of mutant mice from 1600 parts per billion (ppb) to 4200 ppb (untreated normal male littermates: 9100 ppb). Neuronal histopathology between treated and untreated mutant pups was comparable, although treatment extended the lifespan of mutant males by 7% [13.3 days untreated (n=9) vs. 14.3 days treated (n=13); p0.05]. These data indicate that ICV adminstration of copper results in distribution of copper throughout the atp7amo-br brain and increases total brain copper levels. While dose refinement may be needed to optimize outcomes, ICV copper administration ultimately may be a useful approach for rescue of the atp7amo-br mouse, and ultimately for treating classical Menkes patients with severe mutations.

Germline mutation of *SMARCB1* is associated with both syndromic and non-syndromic atypical teratoid rhabdoid tumors (ATRT). *F. Bernier[1], O. Caluseriu[1], E. Payne[2], L. Lafay-Cousin[2,3], D. Strother[2,3], M. Somerville[4], B. McInnes[1]* 1) Department of Medical Genetics, Calgary, AB, Canada; 2) Deparment of Pediatrics, Calgary, AB, Canada; 3) Department of Oncology, Calgary, AB, Canada; 4) Department of Medical Genetics, Edmonton, AB, Calgary.

Atypical teratoid rhabdoid tumors (ATRT) represent 1-2% of pediatric brain tumors. Germline mutation of *SMARCB1/INI1*, a tumor suppressor, results in early predisposition to ATRT as well as multiple schwannomatosis. We present two new patients with ATRTs with different clinical presentations and mutational mechanisms. Patient 1 is male first seen at 11 months of age for dysmorphic features including plagiocephaly, subtle hemifacial microsomia, a hypoplastic right ear, bilateral preauricular skin tags and a left eye epibulbar dermoid cyst and on the basis of these features was diagnosed with Goldenhar syndrome. At 12.5 months of age he presented with neurological deficits due to a large intraaxial mass in the left frontal lobe. Pathology identified a possible glioblastoma multiforme but rhabdoid features and negative immunohistochemical staining for INI1 confirmed a diagnosis of ATRT. FISH in both tumor tissue and cultured skin fibroblast showed a hemizygous deletion of the BCR locus and MLPA confirmed the presence of a constitutional deletion spanning the INI1 locus between markers LZTR1 and SNRPD3 (minimum size of 2.37Mb, maximum of 3.6Mb). Patient 2 is a non-dysmporphic male who presented at 14 months of age with increased head circumference, irritability, and focal neurological signs. A CT scan revealed a mass extending from the 3rd ventricle into the lateral ventricles and hydrocephalus. Tumor pathology and INI1 immunohistochemistry were again consistent with an ARTR. Sequence analysis of SMARCB1 identified a mutation (c.805delC) in exon 7. These patients highlight the need to perform careful clinical and molecular investigations of patients with ATRTs in order to identify the molecular mechanism. In addition this is the second case of a patient with Goldenhar syndrome and microdeletion in the 22q11 region further supporting that this genomic region is a candidate region for Goldenhar syndrome.

Whole genome scan of salt-sensitivity and response to diuretic treatment in essential hypertension. *C. Barlassina[1], C. Cosentino[1], L. Citterio[3], C. Lanzani[3], L. Zagato[3], N. Casamassima[3], M. Simonini[3], E. Salvi[1], F. Torri[1], S. Lupoli[4], P. Manunta[3], G. Bianchi[3], L. Milanesi[5], F. Macciardi[1], D. Cusi[1,2]* 1) Sci & Biomedical Technol, Milan Univ, Milan, Milan, Italy; 2) Division of Nephrology, San Carlo Borromeo Hospital, Milan; 3) Nephrology, Dialysis and Hypertension, Vita-Salute San Raffaele University, Milan; 4) INSPE San Raffaele Scientific Institute Milan; 5) ITB. CNR, Milan.

Introduction: Hypertension is caused by a primary increase of kidney Na reabsorption in at least a subgroup of essential hypertensive patients (EH), also if the underlying genetics is far from being clarified. We addressed this issue with a GWA study in two experimental settings: 1) acute Na-sensitivity test and 2) chronic diuretic treatment. Methods: Illumina HumanHap 300-Duo BeadChips. Plink used for quality control of genotype data, single marker quantitative association and permutations. 1) 326 EH infused with 2L saline i.v. in 2 hrs, after 2 weeks of controlled Na intake (150 mMol/24h), BP recorded over the infusion and recovery (2 more hrs). Phenotype: the difference between mean BP at baseline and after 2 hrs of recovery. 2) 177 EH treated with 25 mg Hydrochlorothiazide UID as monotherapy for 2 months. BP measured throughout the treatment period. Phenotype: the difference between basal mean BP and mean BP after 2 months treatment. Results: 1) 98.3% of SNPs passed QC (mean individual call rate 98.4%). Significant association (p=8x10^-5) was found for SNP rs12064256 in NECAP2 a gene involved in the endocytosis process and expressed in the kidney. It is intriguing to remark that cells expressing the alpha-adducin 460Trp allele display reduced Na/K pump endocytosis as a major pathogenetic event of Na-sensitivity. 2) 99.4% of SNPs passed QC (mean individual call rate 98.8%). Significant association (p=4x10^-5) was found for SNPs rs1531916 in SLC12A1 the kidney specific bumetanide sensitive Na-K-Cl cotransport (2 more SNPs significant within the same gene at p 10^-5). These findings suggest that SLC12A1 may harbor a mutation involved in Na/volume-dependent hypertension.

The power of a multivariate trait-specific test for linkage in complex disorders with multiple quantitative traits.
S. Han, J. Chang Statistics, Yale University., New Haven, CT.

This paper compares a multivariate trait-specific test to a univariate and a more traditional multivariate joint null hypothesis test for detecting linkage for complex disorders with multiple quantitative traits. Multivariate joint null hypothesis tests, which test the effect of a main gene on multiple traits simultaneously, have been found to be more powerful than univariate tests by several studies so far. However,some recent papers have also reported decreased significance using this test, when including a large number traits (3) in multivariate models. A multivariate trait-specific test, proposed by Marlow et al., drops a single variance parameter to test the marginal effect of a main gene on a given trait, in the presence of other traits in a multivariate model. Although this test has been successfully applied to data by several other papers, no systematic evaluation of this test has yet been performed. We first establish the asymptotic null distribution of the multivariate trait-specific test, which needed to be clarified in view of the nonstandard boundary conditions inherent in variance components testing in this multivariate model. We show that the asymptotic null distribution is a chi-square with one degree of freedom regardless of the number of traits in the model. Then we investigate the power of this test, comparing it to both univariate and multivariate joint null hypothesis tests. To characterize the power of all of tests being compared, we show how to evaluate their noncentrality parameters. We find that the noncentrality parameter of the multivariate trait-specific test for a given trait is a function of the genetic effects on the other traits in the model as well as the genetic effects on the given trait and correlations among these effects. Increasing the number of traits in the model having small genetic effect sizes substantially lowered the power of the multivariate joint null hypothesis test due to the sharp increase of its critical values, while the multivariate trait-specific test, with critical values remaining constant over the increase of the number of traits, was less vulnerable to losing its power.

Weaver syndrome (WSS): NSD1 sequence variations, phenotypic spectrum, and natural history, in five new patients. *M. Giovannucci Uzielli, M. Ottaviani, S. Guarducci, E. Lapi, G. Scarselli, L. Giunti* Dept. of Paediatrics, Genetics and Molecular Medicine, University of Florence, 13 Via Luca Giordano, 50132 Florence, Italy.

In 1974, Weaver et al. described two unrelated children with accelerated growth, advanced bone age and a characteristic facial appearance. The disorder subsequently became known as the Weaver-Smith syndrome (WSS): it is characterized by the same clinical features as Sotos syndrome (SS) and other overgrowth disorders (pre-natal and post-natal overgrowth during childhood, development delay, and advanced bone age), but has its own distinctive facial gestalt, and other peculiar features. The range of clinical features associated with WSS is broad and the phenotype is especially characteristic at the facial, fingers, and knee levels. More than 100 distinct intragenic mutations in NSD1 have been identified. NSD1 gene microdeletions or intragenic mutations are present in 60-80% of SS, whereas intragenic mutations are associated with less than one on three WSS subjects. All our patients show broad forehead, round face in infancy, hypertelorism, prominent and large philtrum, micrognathia, with everted lower lip, rounded cheeks, large ears, large hands with deep-set nails and camptodactyly, progressively overriding toes. Flexion at knee level, and limitation of elbow joints extension, are obvious since the infancy. Progressive scoliosis is a constant feature. Variable delays in cognitive, language, and motor development are also observed in all our patients, at any age. No biochemical or endocrinological markers, no cytogenetic abnormalities have been documented in any of the five subjects. We identified 12 new different NSD1 sequence variations in our five, new non-related patients with typical WSS clinical phenotype. Molecular analysis in parents of patients is in progress, in order to better define the nature of the NSD1 sequence alterations.

A Rare Familial Duplication of Xp22.31 that Adds to Characterization of Phenotype-Genotype Correlations of the Short Arm of the X Chromosome. *N. Shur[1, 2], M. Vecchiotti[1, 2], K. Reddy[3], S. Gunn[3], D. Abuelo[1,2]* 1) Hasbro Children's Hospital, Lifespan, Providence, RI; 2) Warren Alpert Medical School of Brown University, Providence, RI; 3) Combimatrix Molecular Diagnostics, Irvine, CA.

Background: Duplications on the short arm of the X chromosome have been rarely reported in males with variable mental retardation (MR) and dysmorphism. Female duplication carriers have been described as normal. Clinical characterization and stratification of affected males, based on aCGH, has initiated the process of phenotype-genotype mapping of the Xp arm. We report a novel familial Xp22.31 duplication, which is likely a reciprocal duplication to the STS deletion, frequently involved in the XX sex reversal exchange between the X and Y chromosomes. Since the duplication is in a highly recombinant region, it may be more common than previously suspected. Case report: Our patient was referred at the age of 3 ½ years because of dysplastic pinnae and behavioral problems, which were increasingly noted during his second year. On physical examination, his head circumference was 52.5 cm (25-50%); weight was 39.5 lbs (less than 3rd percentile); and height 36 cm (< 3rd %). He had short stature, a prominent forehead, simple ears, and hypernasal speech. Velopharyngeal insufficiency was confirmed by videofluoroscopy. A developmental evaluation demonstrated difficulties in fine motor skills and sensory processing. A genetic etiology was suspected: oligoarray on the 44k platform revealed a gain of ~1.5Mb. The genes included in the duplication were VCX3A, HDHD1A, STS, VCX and PNPLA4. The mother was found to be a carrier of the same duplication. Discussion: Previously, cases of males with Xp duplications who had abnormalities were found to be in association with known MR genes on Xp. The genes duplicated in our case (VCX3A, HDHD1A, STS, VCX and PNPLA4) have not been well-characterized, aside from an association of VCX3A with X-linked MR in just one study. We report a novel familial Xp duplication in a patient with sensory processing issues, behavioral problems, and dysmorphic features. Thus, our case offers further insight into the role of various genes on the short arm of the X chromosome.

Modeling ancestry in structured populations using spectral analysis. *K. Roeder, A. Lee, D. Luca* Dept Statistics, Carnegie Mellon Univ, Pittsburgh, PA.

Data for genome-wide association studies are being collected for a myriad of phenotypes. Many of these studies do not include control samples selected to reflect ancestry similar to the case samples. Large "control databases" are becoming available to be utilized as a common resource. How to couple case and control databases effectively is a pressing question. Dissecting ancestry based on principal component analysis (PCA) is the traditional approach to summarizing patterns of ancestry, but a number of problems can arise. PCA is quite sensitive to outliers and fails to detect the key axes of variation when spurious data are present. Moreover, if the sample is ascertained from individuals of disparate ancestries, PCA detects the major axes of variation, but often fails to detect subtle ancestry, such as gradients, present within clusters. As an alternative to PCA, spectral analysis can overcome many of these obstacles. We develop a statistical method to find the axes of genetic ancestry that is not sensitive to outliers and that also discovers major and subtle substructure. We apply these methods to several GWA studies to illustrate the approach in practice.

Characteristics of the Affymetrix 500K GeneChip in African Americans. *L. K. Vaughan[1], A. Patik[1], K. Zhang[1], H. K. Tiwari[1], U. Broeckel[2], D. K. Arnett[3]* 1) Dept Biostatistics, Univ Alabama at Birmingham, Birmingham, AL; 2) Medical College of Wisconsin, Milwaukee, WI; 3) Department of Epidemiology, University of Alabama at Birmingham, Birmingham, AL.

Recent advances in genotyping have opened the door for large scale genome-wide association (GWA) studies to become a powerful tool in the attempt to elucidate the genetic factors underlying complex diseases. Current genotyping platforms have provided valuable information on the genetic architecture of the HapMap populations and their coverage, or ability to capture genetic variation, has been well studied. However, there is little information on the performance of the current genotyping platforms in non-HapMap populations such as African Americans (AA). In such populations coverage cannot be directly estimated. Instead other characteristics such as allele frequencies, linkage disequilibrium, transferability and haplotype structure can be used to evaluate the performance of these platforms in populations other than those represented in the HapMap. We provide a comparison of these characteristics of the Affymetrix 500K GeneChip in the Yoruban (YRI) HapMap individuals with those from an AA population. Although, tagSNPs chosen from the YRI sample are highly portable to the AA population with an average of 93% of the variation in the AA sample captured using YRI tagSNPs, the AA and YRI populations are notably different in terms of the number of mono-allelic markers (0.64% vs 6.62%) and mean r^2 (0.2389 for AA and 0.2528 for the YRI (P 0.0001)). Haplotype analysis also illustrated marked differences between the populations with approximately 60,000 haplotype blocks identified in AA and 50,000 in YRI, with a mean number of SNPs of 6.998 and 8.312 respectively (significantly different at P 0.0001). The average length of the haplotype blocks was also different, with AA haplotypes being shorter (36.2 vs. 44.3 kb) than YRI. Based on our analysis, we expect that the 500K GeneChip will perform as well, if not better, in African Americans when compared to the Yoruban HapMap individuals. This difference may be due in part to the higher coverage for the European component of AA ancestry.

Adsorption of proteins analysis on chemical modified alginate-based capsules for cellular gene therapy. *F. Shen[1], R. Cornelius[1], M. Mazumder[2], J. Brash[1], H. Stover[2], M. Potter[1]* 1) Pathology & Molecular Medicine, McMaster University, Hamilton; 2) Chemistry, McMaster University, Hamilton.

Calcium alginate hydrogel beads layered with poly-L-lysine (PLL) and alginate (APA capsules) for immunoisolation of genetic cells secreting therapeutic proteins have been applied in the treatment of mouse models of disorders such as dwarfism, lysosomal storage diseases, and hemophilia. However, the reliance on calcium cross-linking for capsule stability limits their application for long-term human use. Therefore, incorporation of a covalently cross-linked network in alginate-based capsules was developed. We used a polyanion (designated A70) in the capsules, either as part of the core alginate bead (designated composite capsule) or as part of the coating process (designated layer-by-layer capsule), to react with the polycaton layer (PLL or C70) to form a covalent polymer network. Changes in the chemical composition of capsules were assessed for their effect on biocompatibility, an important component of which is binding of possible immunogenic or pro-inflammatory proteins. The adsorption of proteins from human plasma to the capsule was analyzed using SDS-PAGE and immunoblotting techniques. In this study, four kinds of capsules were investigated: A) APA capsules; B) high PLL APA capsules; C) layer-by-layer capsules; and D) composite capsules. Capsules were incubated in human plasma, and then rinsed 16 times with normal saline before treatment with sodium dodecyl sulfate solution to extract the bound proteins. SDS-PAGE and immunoblotting for 21 different proteins showed that the major species adsorbing to all the capsules were albumin, transferrin, IgG , prothrombin, C3, and vitronectin. However, capsules B and D had higher protein bound. The ratio of total protein bound A:B:C:D was 2:3:2:10. These results suggest that increasing PLL content of the capsules leads to a quantitative increase in bound protein. This model of protein adsorption will be a useful in vitro screening test to identify potentially problematic protein interactions before in vivo studies are conducted.

Prospective evaluation of kidney function in Autosomal Recessive Polycystic Kidney Disease/Congenital Hepatic Fibrosis (ARPKD/CHF). *M. Gunay-Aygun[1,2], L. Lukose[1], K. Drayanani[3], J. Graf[3], J. Bryant[1], A. Garcia[1], D. Adams[1], E. Johnson[1], L. Guay-Woodford[4], P. Choyke[5], W. A. Gahl[1,2]* 1) Section on Human Biochemical Genetics, Medical Genetics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD; 2) Intramural Program of the Office of Rare Diseases, DHHS, Washington, DC; 3) NIH Clinical Center, Bethesda, MD; 4) University of Alabama, Birmingham, Al; 5) Molecular Imaging Program, NCI, Bethesda, MD.

The effectiveness of targeted therapies (vasopressin 2 receptor inhibitors, somatostatin analogs, mTOR (mammalian Target Of Rapamycin) inhibitors, src-inhibitors) in preventing progression of kidney and/or liver disease in animal models of PKD/CHF highlights the importance of identifying disease-specific outcome parameters. In ADPKD, kidney volume is useful as an outcome parameter as it correlates negatively with glomerular function. Such data are not available for ARPKD/CHF, which differs from ADPKD with regard to its age of onset, and the nature of the kidney and liver disease. Between November 2003 and August 2008, we prospectively evaluated 85 probable ARPKD/CHF patients at the NIH Clinical Center (ClinicalTrials.gov, number, NCT00068224). We analyzed the cross sectional and longitudinal data of the 65 patients with at least one mutation in PKHD1, some followed for up to 5 years. Kidney volume in ARPKD/CHF was increased at birth; however, the rate of increase was much slower than for ADPKD. The weighted mean rate of decline in creatinine clearance was 4.0 ml/min/1.73m2 per year; in contrast to ADPKD, this decline rate did not increase with age or kidney volume. Glomerular dysfunction and the urinary concentration defect were significantly worse in patients with both medullary and cortical involvement compared to those with cystic changes limited to the renal medulla. Serum vasopressin was elevated despite dilute urine suggesting resistance to vasopressin. Longer follow up of more ARPKD/CHF patients, along with safety and efficacy data from the ongoing treatment trials on adults with PKD, should enable us to design future therapeutic trials for ARPKD.

Paternal uniparental disomy for the X chromosome causing homozygosity for a large *FMR1* premutation in a female with recurrent miscarriages. *K. J. Friedman[1], E. M. Williams[2], B. L. Williford[4], K. G. Schoch[2], L. L. Oliviero[3], J. H. Tepperberg[4], P. R. Papenhausen[4]* 1) Mol Genetics/CMBP, LabCorp, RTP, NC; 2) Genetic Services/CMBP, LabCorp, RTP, NC; 3) NJ Perinatal Assoc, St Barnabas Med Ctr, Livingston, NJ; 4) Cytogenetics/CMBP, LabCorp, RTP, NC.

Fragile X gene (*FMR1*) premutations are implicated in female reproductive deficits. A 27 year old female with recurrent miscarriages was evaluated for both cytogenetic and *FMR1* abnormalities. A single FMR1 premutation allele of 127 CGG repeats was detected by both PCR and Southern blot. Turner syndrome was ruled out by a normal karyotype as well as the absence of compatible clinical findings. An assessment of consanguinity was addressed by family studies. The patients father is mosaic for premutation alleles of 105 and 136 repeats, while her mother carries two normal range alleles of 20 and 39 repeats. The patients healthy brother received 39 repeats, while a younger sister with a "strange affect" is a premutation carrier, with alleles of 20 and 115 CGG repeats. All have normal karyotypes. The possibility of a small *FMR1* gene deletion on a maternally derived X was evaluated with a comparative genomic hybridization (CGH) array which interrogates 607 submicroscopic loci, including one locus within the Fragile X gene. No deletion was detected. In the absence of consanguinity or detectable chromosomal abnormalities, uniparental disomy studies were performed. Six microsatellite markers on the X were studied. Two markers (DXS559 at location Xq12, and DXS1062 at Xq26) confirmed the absence of a maternal X contribution. A second whole genome copy number cytogenetic array analysis targeting 262,000 single nucleotide polymorphism (SNP) sites (Affymetrix; array also measures DNA dosage at each locus) was performed. The patient had normal dosage at each locus, with complete homozygosity at all loci evaluated, in contrast to the 38% heterozygosity that is typically seen. These data taken together suggest her X chromosomes are paternal duplicates. This paternal uniparental isodisomy for the X likely resulted from paternal meiosis II non-disjunction error (duplicate X) in conjunction with maternal X chromosome loss.

Accurate and Flexible Power Calculations on the Spot for any study design. *H. Tiwari[1], T. Birkner[1], A. Moondan[2], S. Zhang[3], G. Page[1], D. Allison[1,4]* 1) Department of Biostatistics, Section on Statistical Genetics, Univ Alabama at Birmingham, Birmingham, AL; 2) Indian Institute of Technology, New Delhi, India; 3) Department of Statistics, Texas A&M University, Kingsville, TX; 4) Clinical Nutrition Research Center, Univ Alabama at Birmingham, Birmingham, AL.

Often we need to calculate power to demonstrate feasibility of any genetic study for a proposal to NIH or other government agency. Also, power is an essential tool to show superiority of a particular test statistic used in a method over other available methods. There are many tools available to calculate power based on closed formulae following asymptotic theory where the null distribution follows known distribution and the inverse of the distribution can be easily derived. However, in some situations it may not be feasible to derive a closed formula due to the distribution of test statistic follows either a mixture of distribution or unknown distribution and in these situations inverse of the density function may not be possible. In these situations investigator need to perform simulations specific to the study with given parameters such as sample size, mode of inheritance, allele frequency of the disease and marker, etc. In general, most of the investigators depend on collaborators with expertise in statistical genetics either from their own institute or assistance from collaborators from other institutes to perform the simulations to estimate power. In addition, the simulations could be computationally extensive as well as time consuming and could take several weeks to months depending on the nature of the study and may not be available before the proposal deadline. In this manuscript, we provide a simple method to estimate power based on asymptotic theory using other available studies similar to the study of interest to the investigator.

Utilization of high resolution genome-wide oligonucleotide microarrays to detect copy number variations. *M. Nimmakayalu[1], H. Major[1], Q. Qining[1], R. Van Rhedeen[1], D. Hulseberg[1], V. C. Sheffield[1], P. L. Nagy[2,3], S. R. Patil[1]* 1) Dept. Pediatrics, Div. Medical Genetics,; 2) Dept.Pathology; 3) Dept. Biochemistry Univ. Iowa Hospitals & Clinics, Iowa City, IA.

We validated the 385,000 feature comparative genomic hybridization (CGH) oligonucleotide array from Nimblegen-Roche on patients referred for genetic disorders. The array has oligonucleotides representing genes and intragenic regions with an average spacing of 6000bp. The validation studies identified all known macro and micro deletions/duplications and also found 3 novel changes not previously detected with routine karyotyping. These changes were subsequently confirmed by alternative methods. Since the introduction of this platform, we have tested in excess of 124 cases. We found genomic copy number variations (CNVs) not previously described in the normal population in approximately 20% of the cases. Of these, less than half represented changes characteristic of known clinical syndromes while the rest consisted of CNVs not previously described. The smallest deletion detected was 100kb where as the smallest duplication seen was 400kb. Further testing of these cases with the 2.1 million feature array will allow us to precisely identify breakpoints and to design PCR primers to test the parents material for the corresponding changes as well as predisposing inversions. We will present the de novo CNVs found and propose that they represent rare disease associated variants.

Novel MTM1 mutation in a child with myotubular myopathy: Clinical observations with survival to 24 months.

S. Demsey[1], R. Hassan[1,2] 1) Dept of Genetics, Kaiser Permanente Medical Ctr, Bellflower, CA; 2) Dept of Genetics, Kaiser Permanente Medical Ctr, Downey, Ca.

Myotubular myopathy is a heterogeneous disorder characterized by hypotonia, muscle weakness and respiratory distress. The clinical spectrum ranges from a severe neonatal presentation with early death to a mild phenotype with survival up to many decades of life. We report a male infant born at 30 weeks gestation via Caesarean section secondary to premature rupture of membranes, breech presentation, oligohydraminos and a non-reassuring fetal heart rate to a 30 year old primigravida. At birth, the child weighed 1420 grams, did not spontaneously breathe and was intubated and ventilator dependent for first 78 days of life. Extensive metabolic workup, karyotype, DNA methylation testing for Prader-Willi and SMA1 mutation testing was normal. Ultrastructural analysis of skeletal muscle biopsy at 6 months of age revealed a centronuclear myopathy, consistent with myotubular myopathy.

Sequence analysis of MTM1 revealed a transition in exon 13 (c.1388 TC) resulting in substitution of tryptophan for a conserved leucine residue at position 463 (p. L463W). The mother was found to be a carrier of this same previously unreported mutation.

The child is currently 2 years old and has tracheostomy and gastrostomy tubes in place. He has been affected by recurrent pneumonia, is non-ambulatory, nonverbal and requires intermittent ventilator use. His trunk, head, gross and fine motor skills have improved over the course of the last year and he now sits unsupported, has some scooting ability and uses 25 hand signs for communication. The clinical picture of this child with a novel mutation adds to the genotype/phenotype correlation in this disease.

Genome-wide association meta-analysis of 32,000 individuals and replication in 58,000 individuals identifies novel genetic variants associated with body mass index. *C. J. Willer[1], E. K. Speliotes[2], R. J. F. Loos[3], S. Li[3], I. M. Heid[4], C. M. Lindgren[5], GIANT Consortium* 1) Dept Biostatistics, Univ Michigan, Ann Arbor, MI; 2) Broad Institute of MIT and Harvard, Cambridge, MA; 3) Medical Research Council Epidemiology Unit, Institute of Metabolic Science, Cambridge, UK; 4) German Research Center for Environmental Health, Neuherberg, Germany; 5) Wellcome Trust Centre for Human Genetics, Univ Oxford, Oxford, UK.

Obesity is a major and growing public health problem worldwide, resulting in increased morbidity, mortality, and severe economic burdens on health care systems and for individuals. To date, only two common variants (in FTO and near MC4R) have been shown convincingly to impact body mass at the population level. We first conducted a meta-analysis of association results between body mass index (BMI) and ~2.4 million imputed or genotyped SNPs from 13 genome-wide association studies including 32,615 individuals from Germany (KORA), Sweden and Finland (DGI, FUSION), the USA (PLCO, NHS), the UK (WTCCC-T2D, WTCCC-HT, WTCCC-CAD, BC58, NBS and GEM-EPIC), Switzerland (CoLaus), and Italy (SardiNIA). We then attempted replication of 39 promising signals in European-ancestry samples from 14 independent studies (N58,000). Meta-analysis of all GWA and follow-up association results strongly confirms association of FTO ($p = 1 \times 10^{-42}$) and MC4R ($p = 8 \times 10^{-19}$) and identifies six novel loci strongly associated with BMI ($p<10^{-7}$). Non-synonymous SNPs or copy number variants suggest the identity of likely causal genes in several loci, and many of these likely causal genes are highly-expressed in the central nervous system (CNS), including the homologue of a gene implicated in obesity in mice (SH2B1, $p=2\times10^{-10}$, N=83,020), a neuronal growth regulator (NEGR1, $p=6\times10^{-8}$, N=83,660), and a mitochondrial carrier protein (MTCH2, $p=3\times10^{-8}$, N =71,277). Furthermore, it is known that alterations of expression of MC4R and SH2B1 are associated with eating-related phenotypes and that expression of FTO is regulated by feeding and fasting. These results suggest that, as in rare monogenic forms of obesity, the CNS plays a key role in regulation of body mass and common forms of human obesity.

Genome-wide association study of blood pressure in African Americans and Nigerians. *B. Tayo[1], G. Lettre[2,3], S. Kang[4], H. Lyon[2], A. Luke[1], A. Adeyemo[5], C. Rotimi[5], J. Hirschhorn[2,3], X. Zhu[4], R. Cooper[1]* 1) Prev Medicine & Epidemiology, Loyola Univ, Chicago, Maywood, IL; 2) Childrens Hospital Boston, Boston, MA; 3) Broad Institute of Harvard and MIT, Cambridge, MA; 4) Case Western Reserve University, Cleveland, OH; 5) NIH Intramural Center for Genomics and Health Disparities, Bethesda, MD.

Hypertension shares similar heritability with many other traits related to cardiovascular risk, however identifying the genetic variants involved in the etiology of high blood pressure has been difficult. To identify genetic variants influencing high blood pressure among individuals of African origin, we conducted genome-wide association study of systolic and diastolic blood pressure in two separate samples of 735 African Americans and 900 Nigerians without history of use of high blood pressure medication at the time of study recruitment. Association analyses were performed without and with adjustment for sex, age and BMI in both samples, and included 857,989 and 792,581 SNPs genotyped on Affymetrix Genome-Wide SNP array 6.0 in the African-American and Nigerian samples, respectively. Some of the significant (P<1.0E-5) BP-associated variants in these Afro-origin populations are located on known genes such as SLC12A8 and TRERF1 while others are found in regions not yet annotated. Our results present evidence of significant association of BP with a number of genetic variants in regions across the human genome.

Mutations in *TMPRSS6* Cause Iron-Refractory, Iron Deficiency Anemia. *K. E. Finberg[1], M. M. Heeney[2], D. R. Campagna[3], M. D. Fleming[3], N. C. Andrews[4]* 1) Dept Pathology, Duke Univ Med Ctr, Durham, NC; 2) Div Hematology/Oncology, Children's Hosp Boston, Boston, MA; 3) Dept Pathology, Children's Hosp Boston, Boston, MA; 4) Depts Pediatrics and Pharmacology & Cancer Biology, Duke Univ Med Ctr, Durham, NC.

We and others have ascertained kindreds in which multiple individuals exhibit iron deficiency anemia unresponsive to oral iron therapy but partially responsive to intravenous iron, a phenotype we term Iron-Refractory, Iron Deficiency Anemia (IRIDA). Using a positional candidate approach, we have found that IRIDA is caused by mutations in *TMPRSS6*, located at 22q12.3, which encodes a type II transmembrane serine protease primarily expressed by the liver. Sequence analysis of *TMPRSS6* coding regions and intron/exon boundaries revealed frameshift, nonsense, splice junction, and non-conservative missense mutations in 5 multiplex IRIDA kindreds and 2 sporadic IRIDA cases; the disease-associated variants were absent in public SNP databases and in 100 control chromosomes. In 3 of these 5 multiplex kindreds, we identified biallelic mutations; however, in affected individuals from the 2 other kindreds (which were compatible with linkage to 22q12.3), a single heterozygous mutation was identified. In these 2 kindreds, *TMPRSS6* 5 and 3 UTR sequences and *TMPRSS6* exon copy number, as assessed by multiplex ligation-dependent probe amplification, were normal, suggesting that the parental chromosome 22 on which a *TMPRSS6* mutation was not detected harbors a genetic alteration outside of the *TMPRSS6* coding and UTR regions that results in impaired TMPRSS6 function. Urinary levels of hepcidin, a peptide hormone produced by the liver that downregulates intestinal iron absorption and macrophage iron release, were inappropriately elevated in 5 IRIDA cases from 3 kindreds examined, suggesting that TMPRSS6 normally functions to downregulate hepcidin expression. In summary, our results reveal a novel regulatory role for TMPRSS6 in systemic iron homeostasis in humans. Clinical testing for *TMPRSS6* mutations may be complicated by the existence of genetic alterations outside of the coding and 5 and 3 UTR regions in some IRIDA cases.

Association and homozygosity mapping for loci contributing to type 2 diabetes on the Pacific island of Kosrae. *J. B. Maller[1,2,3], J. K. Lowe[1,2,4], B. M. Neale[1,2,5], J. Salit[4], J. L. Breslow[4], M. Stoffel[4], M. J. Daly[1,2,6], D. M. Altshuler[1,2,6], J. M. Friedman[4,7]* 1) Broad Institute of Harvard & MIT, Cambridge, MA; 2) Massachusetts General Hospital, Boston, MA; 3) Department of Statistics, University of Oxford, Oxford, United Kingdom; 4) Rockefeller University, New York, NY; 5) Kings College, London, UK; 6) Harvard Medical School, Boston, MA; 7) Howard Hughes Medical Institute.

We performed association and homozygosity mapping in an inbred population from Kosrae, Federated States of Micronesia, to identify risk loci for type 2 diabetes. As observed in other population isolates (eg, Pima Indians), native Kosraens exhibit markedly increased prevalences of type 2 diabetes and obesity as compared to Caucasians. Approximately 33% of Kosraen adults age 40-60 are diabetic, compared to ~10% of US adults of similar age. The type 2 diabetes and obesity epidemics on Kosrae are thought to result from rapid adoption of a Western lifestyle, and suggest an enrichment of genetic susceptibility factors in this population. The Kosrean population shows reduced genetic diversity from a strong founder effect, geographic isolation and severe population bottlenecks. Consequently, we hypothesize the existence of comparatively few genetic variants of larger effect underlying disease in this cohort as compared to Caucasian populations. Approximately 75% of Kosraen adults were ascertained over population-based screens in 1994 and 2001. A total of 2906 individuals were successfully genotyped with the Affymetrix 500k platform, of whom 2842 individuals can be joined in a single extended pedigree. We developed analytic methods for identification of common variants via genome-wide association analyses, and rare variants via homozygosity mapping in this highly related cohort. We compare our findings to true associated variants identified in Caucasian populations. Our results suggest that common variants of small effect underlie type 2 diabetes on Kosrae.

Reciprocal Co-regulation of *EGR2* and *MECP2* is Disrupted in Rett Syndrome and Autism. *S. E. Swanberg, R. P. Nagarajan, D. H. Yasui, S. Peddada, J. M. LaSalle* Medical Microbiology and Immunology, University of California Davis, Davis, CA.

Mutations in *MECP2* encoding methyl CpG-binding protein (MeCP2) cause the neurodevelopmental disorder Rett syndrome (RTT) and MeCP2 rxpression defects are frequent in autism brain. MeCP2 is a neuronal epigenetic factor important in modulating gene expression, including expression of the activity-dependent genes *Bdnf* and *JUNB*. Another activity-dependent gene, Early Growth Response Gene 2 (*EGR2*), which plays a role in both early hindbrain development and mature neuronal functioning, has putative binding sites in the 5' regulatory regions of a number of neurologically relevant genes including *MECP2*. Conversely, MeCP2 family members, MBD1, MBD2 and MBD4 bind a methylated CpG island with enhancer-like properties, located in *EGR2* intron 1. This study was designed to test the hypothesis that *MECP2* and *EGR2* regulate each other's expression during neuronal maturation in postnatal brain development. Downregulation of *EGR2* and *MECP2* in cultured human neuroblastoma cells by RNA interference reciprocally reduced expression of both genes and their protein products. Chromatin immunoprecipitation analysis showed Egr2 binding to the *MECP2* promoter and MeCP2 binding to the enhancer region in *EGR2* intron 1. Consistent with a role for MeCP2 in enhancing *EGR2*, *Mecp2*-deficient mouse cortex samples showed sinificantly reduced Egr2. Furthermore, RTT and autism postmortem cortex samples showed significant reduction of EGR2 compared to controls, supporting the involvement of an MeCP2-EGR2 co-regulated pathway. Taken together, these data suggest dysregulation of the reciprocally-regulated factors MeCP2 and EGR2 in RTT and autism.

A Searchable Database of Genetic Evidence for Psychiatric Disorders. *T. Konneker[1], T. Barnes[1], H. Furberg[1], M. Losh[2], C. M. Bulik[3], P. F. Sullivan[1,3]* 1) Department of Genetics, University of North Carolina at Chapel Hill, Chapel Hill, NC; 2) Department of Allied Health Sciences, University of North Carolina at Chapel Hill, Chapel Hill, NC; 3) Department of Psychiatry, University of North Carolina at Chapel Hill, NC.

Background. We have developed a new bioinformatics tool called SLEP (Sullivan Lab Evidence Project). SLEP was created to contextualize the relevant findings from the many psychiatric genetic studies. SLEP is a searchable archive of findings from psychiatric genetics that is freely available on the web for non-commercial use (http://slep.unc.edu). Methods. More than 150 papers have been identified for SLEP through overlapping Pubmed searches and citations. All studies with a genome-wide focus and clear phenotypic definitions were included. These studies included genomewide linkage, genomewide association, and microarray studies. Two raters reviewed each paper and recorded significant findings (GWL LOD 1.5, GWA p-value <0.05, MA p-value <0.05). Results. Via a simple interface, users can retrieve findings from GWL, GWA, and MA for ADHD, autism, bipolar disorder, eating disorders, major depression, nicotine dependence, and schizophrenia. Users can search for findings by gene name, genetic markers, and chromosome region. Results are displayed in summary form and include relevant study information and links to PubMed and the UCSC Genome Browser. SLEP includes a database of genetic signposts (i.e., physical genomic features, confirmed associations from complex human diseases, genes with evidence of imprinting, selection, or monallelic expression, SNPs associated with gene expression differences in human cortex, and copy number variants and inversions from the Database of Genomic Variants. The SLEP database will be updated quarterly. Conclusions. Given that limited power has been a concern for most psychiatric genetic studies, SLEP will likely turn up false positives. With that in mind, SLEP should be treated as a tool for searching and integrating findings from psychiatric genetics research. It will be useful for researchers looking to place new empirical findings in the context of prior studies.

Genome-wide association study of amyotrophic lateral sclerosis identifies multiple loci. *B. J. Traynor[1,14], A. Chio[2], J. C. Schymick[1,15], G. Restagno[3], S. Lai[1], G. Mora[8], L. Ferrucci[4], F. Macciardi[5], S. J. Chanock[6], C. Gieger[7], H. E. Wichmann[7], J. Connor[10], T. Dunkley[12], D. A. Stephan[12], M. Sendtner[11], M. Beck[11], L. Bruijn[13], J. Rothstein[14], A. Singleton[1], J. Hardy[1,9]* 1) Laboratory of Neurogenetics, NIA, NIH; 2) Department of Neuroscience, University of Turin; 3) Molecular Genetics Unit, Department of Clinical Pathology, A.S.O. O.I.R.M.-S.Anna, Turin; 4) Longitudinal Studies Section, NIA, NIH; 5) Department of Science and Biomedical Technology, University of Milan; 6) Division of Cancer Epidemiology and Genetics, NCI, NIH; 7) Institute of Epidemiology, Neuherberg/Munich, Germany; 8) Salvatore Maugeri Foundation, Lissone, Italy; 9) Institute of Neurology, Queen Square, London, UK; 10) Department of Neurosurgery, Penn State College of Medicine; 11) Institute of Clinical Neurobiology, University of Wuerzburg, Germany; 12) Neurogenomics Division, TGEN, Phoenix, AZ; 13) The ALS Association, Palm Harbor, FL; 14) Department of Neurology, Johns Hopkins University, Baltimore; 15) Department of Physiology, Anatomy and Genetics, University of Oxford, UK.

BACKGROUND: We sought to identify genetic variants associated with an increased or decreased risk for developing ALS in a cohort of sporadic cases. **METHODS:** We undertook a two-stage GWAS: we followed our initial GWAS of 545,066 SNPs in 553 individuals with ALS and 2,338 controls of European descent by testing 7,600 SNPs in three independent cohorts consisting of 2,160 cases and 3,008 controls. **FINDINGS:** We identified four highly significant loci on chromosomes 3, 7 (two loci) and 11, three of which exceeded Bonferroni correction for multiple testing (P value between 1.17x10-5 and 3.55x10-6). The locus on chromosome 3p13 includes RYBP, a member of the apoptosis pathway. Loci on chromosome 7 include SUNC1, a nuclear envelope protein; and CNTNAP2, which regulates clustering of voltage-gated potassium channels at juxtaparanodal regions of myelinated axons. The fourth locus is located near CD82. **INTERPRETATION:** Our findings suggest that multiple loci with moderate effects are associated with susceptibility to sporadic ALS.

Characterization of common human copy number variants with oligonucleotide arrays. *A. Ben-Dor, P. Anderson, N. Sampas, A. Tsalenko, S. Giles, P. Tsang, A. DeWitte, D. Bailey, Z. Yakhini, S. Laderman, L. Bruhn* Agilent Technologies, Santa Clara, CA.

Technological improvements for efficient genotyping of CNVs over a broad dynamic range of copy number are important for genetic studies of normal and disease-related human variation. We are developing microarray-based methods and data analysis tools to identify CNV regions and distinguish their copy number states. We employ two complementary statistical methods to determine the copy number state of a particular CNV. The first approach is based upon direct correlation of CNV copy number to its signal intensity level. The second approach aims at identifying distinct clusters of copy number states across the cohort for each individual CNV region. In order to examine the systems capability for discriminating copy number states, we used an oligonucleotide array set comprised of two 244K feature arrays enriched for coverage of 19,400 previously identified CNV, in-del and segmental duplication regions. We employed a non-enzymatic labeling protocol that is amenable to high-throughput applications, to measure 27 HapMap samples in two-color hybridizations with a common reference sample (NA15510). We detected an average of 841 CNVs per sample as compared to an average of 6 calls in 3 self-self experiments. Of 1326 CNV regions called in two or more individuals, 817 (60%) have consistent breakpoints (+/- 1 probe). The remaining CNV regions have boundaries that vary between individuals, in some cases reflecting what appear to be complex structures as observed in previous studies. Based on the robust separation of signal intensity levels, we call distinct copy number states for 0, 1, and 2 copies. On average, approximately 5% of the CNVs detected are homozygous deletions, 25% have 1 copy, 25% have 2 copies, and 45% have 3 copies or more. Employing a cohort based approach, we identify many regions with clear separations of the samples into distinct copy states including some with 4 or more copy number states. We are exploring the appropriate interpretation of the signal and ratiometric measurements in these regions as a foundation for more broadly ascertaining copy number states of CNVs.

The A-844G Polymorphism in the PAI-1 gene is associated with susceptibility in systemic lupus erythematosus. *J. R. Padilla-Gutiérrez[1,2], C. A. Palafox-Sánchez[1], N. M. Torres-Carrillo[1], Y. Valle[1,2], G. Orozco-Barocio[3], I. García-De la Torre[3], N. Torres-Carrillo[1], M. Vázquez Del Mercado[1], J. F. Muñoz-Valle[1]* 1) IIRSME, C.U.C.S, UdeG; 2) Posdoctoral Fellow in Biomedical Sciences (Immunology), UdeG; 3) HGO, SSJ.

Introduction. Systemic lupus erythematosus (SLE) is a systemic autoimmune disease characterized by the presence of autoantibodies against nuclear autoantigens as well as cytoplasmic and circulating proteins. The thrombotic phenomena in SLE is associated in a 10-20%. Recent studies indicate that endothelial cells play an important role in fibrinolysis by secreting both tissue-plasminogen activator (t-PA) and plasminogen activator inhibitor-1 (PAI-1), which regulates the activity of t-PA. In fact, it is known that the expression of the PAI-1 is modulated by proteins such as cytokines. Several studies have suggested that in patients with systemic diseases, the fibrinolysis may be decreased, due mainly to an excess of PAI-1. Methods. We enrolled 71 SLE patients classified according to ACR criteria and 71 healthy subjects (HS). The A-844G PAI-1 polymorphism was identified by PCR-RFLP. The DNA fragments were observed on a 2% agarose gel staining with ethidium bromide. The statistical analysis was performed using MedCalc Statistical Software. Results. The allele A of PAI-1 polymorphism (A-844G) showed a significant difference in SLE patients (41%) compare with HS (27%) [p= 0.01; OR=1.8, 95%, CI=1.1-3.0]. In addition, the A-844G PAI-1 polymorphism was associated with increased risk for SLE in a dominant model (OR= 2.2, 95% CI=1.14-4.44). Conclusions. The A allele of the A-844G PAI-1 polymorphism might be an additional risk factor for the development of SLE in Mexican population.

Microarray- DNA Sequence Capture of the Human Exome in a Family Identifies Spontaneous Mutations in the Human Germline. *M. N. Bainbridge[1,2], T. J. Albert[3], D. M. Muzny[1], L. Nazareth[1], C. M. Middle[3], T. A. Richmond[3], M. J. Rodesch[3], M. Egholm[4], R. A. Gibbs[1]* 1) Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX; 2) Program for Structural and Computational Biology and Molecular Biophysics, Baylor College of Medicine, Houston, TX; 3) NimbleGen Systems Inc., Madison, WI; 4) 454 Life Sciences Corp., Branford, CT.

Spontaneous mutations in the human germline are of central importance in genetics and evolution. Their rate of occurrence has been estimated by indirect methods to be 100-200/genome/generation, or ~5 amino acid changing mutations. We used DNA capture and sequencing (via Roche/NimbleGen/454 Life Sciences technology) of the entire exon set (exome) of a family to discover new mutations. We found 7 occurrences among ~7.5 million scanned bases and calculated these correspond to an exonic spontaneous mutation rate of 33-45/generation-exome, a higher value than previously thought. As expected, approximately 40% of the new mutations are transitions in CpG residues. In addition, the other single base mutations within exons do not occur randomly, but appear to preferentially occur at sites already known to be polymorphic. While additional families (meiosis) should be studied, these findings already support finite-site models for human mutation and have implications for human evolution.

Allele and genotype sharing in large databases: reconciliation of observed and expected statistics. *R. Chakraborty, J. Ge, H. S. Lee* Center for Genome Information, Univ Cincinnati, Cincinnati, OH.

Using a variety of panels of genetic markers, several large databases in various contexts have been created and curetted for different purposes. Population- or family-based multilocus genotype profiles of cases and control subjects involving genome-wide panel of genetic markers are commonly used for mapping genes for disease traits as well as phenotypes that are influenced by genetic factors. In DNA forensics, large databases of multilocus DNA profiles involving unlinked genetic markers are used for solving crimes where perpetrators are not initially identified by common criminal investigative tools. Allele and genotype sharing distribution between individuals in such databases have recently come under discussion in the context of validity of population genetic assumptions in estimating rarity of any specific multilocus profile. This research starts with a brief overview of this subject. Under the assumption of independent segregation of markers encompassed in the multilocus genotype profiles, algorithms are provided to compute the expected distributions of allele and genotype sharing in such databases, even with complexities of having relatives within such databases and existence of population substructure within the sample. It is shown that for testing the assumptions of independence of alleles within and across loci, reconciliation of the observed and expected distribution of allele and genotype sharing is possible only with complete details known for each individual in the database, including not only their individual multilocus profiles, but also their substructure affiliation and pairwise relatedness with others at individual level. Further, we show that the expected distributions are not necessarily the same using the locus average values as opposed to genotype-specific composition of the database. Finally, we argue that simulation or large-sample approximations do not always provide an accurate approach of reconciliation of observations and expectations based on population genetic models, particularly when the number of markers is large, or majority of them contain large numbers of segregating alleles.

Robin sequence, short and bowed long bones and multiple fractures - A new skeletal dysplasia? *N. Martin[1], J. Kingdom[2], C. Grasemann[3], E. Sochett[3], S. Unger[4], D. Chitayat[1,5]* 1) Prenatal Diagnosis, Mount Sinai Hospital, Toronto, ON, Canada; 2) Obstetrics and Gynecology, Mount Sinai Hospital, Toronto, ON, Canada; 3) Dept. of Endocrinology, Hospital for Sick Children, Toronto, ON, Canada; 4) Institute of Human Genetics, University of Freiburg, Germany; 5) Clinical and Metabolic Genetics, University of Toronto and Hospital for Sick Children, Toronto, ON, Canada.

Prenatal finding of bowed limbs and post-natal fractures is most often seen in osteogenesis imperfecta. Our case demonstrated both of these features but is distinguished by exclusion of type I collagen defects and an unusual fracture pattern. The patient was born to a G2P1L1 mother. Both parents were healthy and non-consanguineous and their family history was non-contributory. Fetal ultrasound at 25 weeks gestation identified short limbs. Follow-up ultrasounds demonstrated bent femurs, apparently normal mineralization, and a narrow chest. Delivery was at 39 weeks gestation via C-section. The birth weight was 2.4 kg (10th percentile), length 45.5 cm (10-25 percentile) and OFC 33.2 cm (25-50 percentile). On physical examination, the child had a long trunk with short limbs (upper/lower segment ration = 2.03), prominent eyes with blue-grey sclera, low set, thin ears, micrognathia, and a narrow chest with pectus carinatum. Skeletal survey revealed hypomineralization of the skull, bell-shaped chest with gracile ribs and healed fractures. All long bones showed flaring and beaking of the metaphysis, patchy mineralization and cortical deformity. Multiple fractures involving the left clavicle, right ulna and tibia were noted. There was also shortening of the metacarpals. He has a duplex right kidney, bilateral hydroceles, and severe gastroesophageal reflux. Mutation analysis of the COL1A1 and COL1A2 genes showed no detectable mutaion. Collagen electrophoresis studies, and electrolytes, karyotype were normal. To the best of our knowledge this is a not yet reported non-lethal skeletal dysplasia associated with osteopenia, multiple fractures and facial dysmorphism.

Mutations in CYP7B1 are responsible for hereditary spastic paraplegia type 5. *C. Goizet[1,2], A. Boukhris[1], A. Durr[1], J. Truchetto[1], L. Guyant-Marechal[3], B. Fontaine[4], D. Grid[1], C. Depienne[1], S. Forlani[1], M. Tsaousidou[5], A. Crosby[5], A. Brice[1], G. Stevanin[1]* 1) INSERM / UPMC U679 - NEB, Paris, France; 2) Med.Genetics, Univ. Victor Segalen Bordeaux 2, CHU Pellegrin, Bordeaux, France; 3) Dept of Neurology, CHU Rouen, Rouen, France; 4) Federation of Neurology, GH Pitié-Salpêtrière, Paris, France; 5) Med. Genetics, St Georgess Univ., London, UK.

Thirty-eight different loci for hereditary spastic paraplegia (HSP) have been mapped, 20 in autosomal recessive spastic paraplegias (AR-HSP). AR-HSPs usually have clinically complex phenotypes but the SPG5, SPG24 and SPG28 loci are considered to be associated with pure orms of the disease. Very recently, five mutations in the CYP7B1 gene, encoding for a cytochrome P450 oxysterol 7- hydroxylase, have been demonstrated in SPG5 families. We analyzed CYP7B1 by direct sequencing in a series of 60 unrelated AR-HSP and 20 sporadic HSP patients, all manifesting a pure form of the disease. We identified 4 different mutations in 4 index cases with AR-HSP, including 3 missense and 1 nonsense mutations. No mutation was found in sporadic cases. The mutations segregated with the disease in all the families and were absent from 494 control chromosomes. We obtained clinical and paraclinical data in a total of 7 patients from these families. The frequency of SPG5 was 6.6% (n=4/60) in our series of AR-HSP families with pure form. We detected a cluster of missensse mutations in the exon 6 at the C-terminal end of the protein. Mean age at onset was 15.9 yrs 12.8 (4-47). Spasticity and functional handicap were noted moderate to severe in all cases (severe, n=5) after mean disease duration of 24.1 yrs 16.5. The recent identification of CYP7B1 as the SPG5 responsible gene highlight a novel molecular mechanism involved in the HSP determinism.

Tracing ancestral lineages, long-range LD, and homozygosity blocks in the genomes of the Qatari. *R. G. Crystal[1], T. P. O'Connor[1], N. R. Hackett[1], L. Chouchane[2], J. Salit[1], J. G. Mezey[3], A. R. Boyko[3], K. E. Lohmueller[4], A. G. Clark[4]*
1) Genetic Medicine, Weill Cornell Medical College, New York, NY; 2) Weill Cornell Medical College in Qatar; 3) Biol. Statistics and Comp. Biology, Cornell Univ, Ithaca, NY; 4) Molec. Biol. and Genetics, Cornell Univ, Ithaca, NY.

People of the Qatari peninsula represent a relatively recent founding by a small number of families from three tribes of the Saudi peninsula, Persia, and Oman. The combination of this founding effect and the customary first-cousin marriages among the ancestral Islamic populations could result in an interesting pattern of LD, shared identity blocks, and runs of homozygosity, all relevant to efforts to map genes associated with complex disorders. Here we obtained DNA samples from 120 self-reported Qatari nationals sampled from Doha, Qatar in the past 12 months, and performed hybridizations to Affymetrix Genome-wide Human SNP array 5.0 to obtain genotypes calls of nearly 500,000 SNPs in each individual. The overall levels of diversity in this sample is close to that of European populations, but the extent of linkage disequilibrium is more extensive, and runs of homozygosity in some individuals reflects impressive consanguinity. The among-individual sharing of long haplotypes is also exceptional in this population, with several multiple-Mbp identity blocks found in multiple individuals. Principal components analysis was performed along with samples from the global collection of the Glaxo-SmithKline PopRes dataset, revealing a cluster of genotypes between the northern African and eastern European samples. Simulations of a population in which one-half the population marries a first cousin generally reflect greater identity-by-descent sharing than was observed, but the discordance is small. Most of these conclusions are robust to the fact that the SNPs of the Affymetrix 500k chip were ascertained with bias toward SNPs common in Europeans. The data strongly support the notion that the Qatari population could provide a valuable resource for the mapping of genes associated with complex disorders, and that tests of pairwise interactions are particularly empowered by populations with elevated LD like the Qatari.

Genome-wide patterns of population structure and admixture in Africans and African Americans. *K. Bryc[1], M. Nelson[2], J. Oksenberg[3], S. Hauser[3], C. Bustamante[1,5], S. Tishkoff[4,5]* 1) Dept Biol Statistics/Comp Biol, Cornell Univ, Ithaca, NY; 2) GlaxoSmithKline, Research Triangle Park, NC; 3) Department of Neurology, University of California, San Francisco, CA, USA; 4) Departments of Genetics and Biology, University of Pennsylvania, Philadelphia, PA, USA; 5) These authors contributed equally to this work.

We present results from a study of high-density genome-wide patterns of variation in Africans, Europeans, and African Americans. Our data set consists of 203 individuals from 12 African populations concentrated in West Africa, 365 African Americans and 400 Europeans from 42 countries, genotyped using the Affymetrix GeneChip 500K Array Set. Arrays passing quality checks were analyzed using both FRAPPE and *smartpca*, a principal component analysis (PCA) method for genotype data. We find that population structure within the African populations coincides with geographic distance and barriers to gene flow, and that the sample clustering on PC1 and PC2 resembles a map of sample origins in Africa.

To facilitate admixture mapping within our African American individuals, we introduce a PCA-based approach for estimating local and genome-wide admixture proportions. Applying our method, we find evidence for admixture of Europeans with several (but not all) of the sampled African populations from West Africa. Furthermore, our results suggest that much admixture in African Americans derives from ancestral populations that are not represented in current studies of African diversity. Overall, we estimate that the median proportion of European admixture among African Americans is 18.5% (inter-quartile range 11.6% - 27.7%) in line with previous estimates. The estimated proportion of European ancestry varies by geographic origin of the African Americans sampled. Comparing the median values of European ancestry of regions in the United States, we find that the proportion of European ancestry is lowest in the South (16.6%) and highest in the West (20.7%) and Northeast (21.0%) regions. Funded by NIH grant 5R01GM083606-02 to CDB and SAT.

Clinical and Molecular-Cytogenetics Characterization of a man with a 14 ring chromosome: a 19-year follow-up.

V. F. A. MELONI[1], F. T. LIMA[1], R. M. MINILLO[1], R. S. GUILHERME[2], C. P. CORREIA[2], L. D. KULIKOWSKI[2], M. I. MELARAGNO[2], D. BRUNONI[2] 1) CENTRO DE GENÉTICA MÉDICA, UNIVERSIDADE FEDERAL DE SÃO PAULO, SÃO PAULO, SÃO PAULO, BRAZIL; 2) DISCIPLINA DE GENÉTICA, UNIVERSIDADE FEDERAL DE SÃO PAULO, SÃO PAULO,SÃO PAULO, BRAZIL.

The 14-human chromosome accounts for over 550 genes and over 30 diseases. Its ring aberration occurs more often in females and encompasses facial dysmorphic features, seizures, developmental delay, hypotonia, growth retardation, retinal pigmentation abnormalities and pulmonary infections. We describe a 22-year-old male who at birth presented bilateral cryptorchidism, sacrum dysgenesis, coccyx agenesis and talo-valgus deformity. At the first evaluation he was a 3-year-old boy with short stature, microcephaly, facial dysmorphisms and mild psychomotor delay. His first GTG-banded karyotype showed 46,XY,r(14)(p13q32) in 300 analyzed cells. Parents were cytogeneticaly normal. Nowadays he has mild mental retardation, short stature, microcephaly and slightly different facial dysmorphisms. He also has a hypopigmented area in posterior pole in both eyes. Cytogenetic reevaluation was done 19 years later and showed 5% of metaphases with aneuploid of chromosome 14 in 200 analyzed cells. FISH using Alpha Satellite probe specific for chromosome 14 was performed according to standard protocols on 100 interphase nuclei of the proband. In 90 cells (90%), the FISH showed a monocentric r(14) chromosome and 2 cells (2%), a dicentric ring containing duplicated short and long arm material of chromosome 14 was observed. Eight metaphases lacked any ring of chromosome 14. The ring chromosome found in this individual shows evidence of the characteristic instability associated with ring chromosomes, such as duplicated segments, double rings, and subsequent loss of the ring resulting in cells with monosomy 14. This abnormalities associated with the clinical follow-up help our understanding of the mechanisms underlying the genotype-phenotype correlation. The long follow-up allows a better knowledge of the natural history of the disorder helping the counseling and management of the patients. Financial support: CAPES, FAPESP.

Defining the etiology of pancreatic cancer: implications for targeted adjuvant therapy and genetic counseling. *S. Charles, S. Showalter, A. Witkiewwicz, J. Cozzitorto, J. Belin, P. Sauter, E. P. Kennedy, C. J. Yeo, J. R. Brody* Thomas Jefferson University Hospital, Philadelphia, PA.

Better comprehension of the molecular pathogenesis of pancreatic ductal adenocarcinoma (PDA) has led to the development of novel targeted treatments and, in some cases, more accurate genetic counseling for PDA susceptibility. We present two cases of PDA: Patient 1 presented with PDA at 71 years in the context of a frameshift mutation (2157delG) in BRCA2, suspected due to her personal and family history of breast cancer. As recent data have shown that PDAs with defects in the BRCA2/Fanconi Anemia pathway are hypersensitive to interstrand cross-linking agents, most notably platinum-based drugs, Patient 1 was treated with gemcitabine and cisplatin, in addition to capecitabine and radiation. She is currently disease free 18 months post distal pancreatectomy. Family members have undergone counseling and testing for the familial BRCA2 mutation, and screening recommendations tailored to their genetic status and family history have been made. Patient 2 presented with PDA at 50 years and had a family history notable for pancreas cancer in her father and paternal cousin, plus pancreatitis in two paternal cousins. Germline genetic studies for patient 2 were not informative, as she did not carry a BRCA1/2 Ashkenazi Jewish founder mutations (i.e., 187delAG and 5385insC in BRCA1 and 6174delT in BRCA2) and she did not carry the novel missense PRSS1 variant identified in a paternal cousin with pancreatitis (i.e., C185Y, a cysteine changed to a tyrosine within exon 4). No molecular targets were identified in DNA repair genes FANCA, FANCC, BRCA2, and BLM, which were analyzed from a non-xenografted PDA cell line. Patient 2 received neoadjuvant gemcitabine and erlotinib. She died 8 months after surgery. Family members have been counseled and offered surveillance under the assumption of familial pancreatic cancer, though etiology remains unclear. These cases highlight the challenge of efficiently selecting patients that will optimally benefit from targeted therapy, as well as the challenges of providing genetic counseling for familial pancreatic cancer when causative genes remain undefined.

Investigation of relationship between NDUFS1 gene with MS disease. *Z. Baratieh Najafabadi[1,2], M. H. Sanati[2], M. Houshmand[3], M. Rezaei-Tavirani[1]* 1) Cellular & Molecular Biology Depatment, Khatam University, Tehran, Iran; 2) National Institute for Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran; 3) Genetic section, Special Medical Center, Tehran, Iran.

Multiple sclerosis (MS) is the most common inflammatory demyelination disease of the central nervous system. Preferential maternal transmission in familial cases and the occasional association of MS and LHON, suggests on involvement of mitochondrion (mtDNA, electron transport chain enzymes) in the aetiology of MS. Besides, in recent study, researchers found a relationship between biochemical defect in complex I enzyme activity and pathogenesis of MS. Complex I is a largest complex of the mitochondrial respiratory chain that contains 43 subunits.36 subunits being encoded by nuclear and 7 subunits being encoded by mitochondrial DNA. As mutations in NDUFS1 gene were reported previously in patients afflicted by complex I deficiency and also biochemical defect in complex I enzyme was found in Iranian MS patients, we encouraged to study relationship between NDUFS1 gene with MS disease in Iranian MS patients. This gene is a nuclear gene encoding catalytic subunit of complex I enzyme. So we analyzed four exons of NDUFS1 gene (8, 9, 15 & 19) that some mutations are identified in patients with complex I deficiency. We amplified these exons by PCR and screened them for finding any variations by SSCP method. Suspicious samples for mutations were sequenced. Finally, in our samples, we couldn't find any variation in these regions of NDUFS1 gene. To identify the relation between NDUFS1 gene and complex I with MS disease, further analyzes should be done on other exons of this gene and also other genes of complex I subunits.

Effects of sequence variation on mtDNA deletion quantification: Ramifications for genetic testing in biomedical research. *S. I. Zhadanov[1,2], T. S. Vierbuchen[1], T. G. Schurr[1]* 1) Anthropology Dept, Univ Pennsylvania, PA; 2) Institute Cytology and Genetics SB RAS, Russia.

It has been proposed that a lifetime accumulation of mtDNA deletions in postmitotic tissues can be an important contributor to the progression of neurodegenerative or cardiovascular disorders and is likely to have a central role in the aging. To quantify the presence of mtDNA deletions at the cellular level in single cells and tissues, many detection methods currently employ Taqman/RT-PCR assays. However, this approach may be biased by the considerable individual- and population-specific genetic diversity of the mtDNA. We examined the effects of polymorphic mtDNA sequence variants (SNPs) in the primer and probe regions frequently used for this analysis. Using Taqman MGB probes, we determined the effects of sequence variation on the RT-PCR reactions. Using absolute quantification RT-PCR, we compared mtDNA derived from cultured cells that contained two SNPs in the primer and two SNPs in probe binding site. Our sample with four total SNPs showed a 16-fold copy number difference for our region of interest, thus, making it indistinguishable from a large scale deletion. We hypothesize that heteroplasmic SNPs in the primer and probe regions can significantly confound the results of these analyses, causing both overestimation and underestimation of the true deletion load. Our findings indicate that common population-specific SNPs reside in the primer and probe regions used for many mtDNA deletion assays, thereby confounding RT-PCR assessments of somatically derived mutation levels. For this reason, researchers should not rely solely on RT-PCR to diagnose mtDNA deletions in single cells or in homogenous tissue samples. Without confirmation from alternative methods, such as sequencing or Southern blotting, it may be difficult to ascertain the exact level of deleted mtDNA within the range of error caused by SNPs in the primer and probe regions. Thus, to appropriately screen tissue samples for mtDNA deletions (esp. at low levels) as biomarkers of oxidative stress or age-related pathologies, one must employ multiple approaches to offset the effects of this genetic heterogeneity.

Interrogation of Folate Pathway Genes and NSCLP. *R. R. Henry[1], J. B. Mulliken[2], S. Stal[3], A. Burt[4], S. H. Blanton[4], J. T. Hecht[1]* 1) University of Texas Medical School at Houston; 2) Children's Hospital, Boston, MA; 3) Texas Children's Hospital; 4) University of Miami Miller School of Medicine.

Nonsyndromic cleft lip with or without cleft palate (NSCLP) is a common birth malformation caused by genetic, environmental, and/or gene-environment interactions. Periconceptional supplementation of folic acid, a key component in DNA synthesis and cell division, has reduced the birth prevalence of neural tube defects and may similarly affect other complex birth defects such as NSCLP. Past studies have yielded conflicting results when evaluating two common polymorphisms, C677T (rs1801133) and A1298C (rs1801131), in the MTHFR (methylenetetrahydrofolate reductase) gene and their relation to NSCLP. In this study, we asked whether variation in other genes in the folate pathway were associated with NSCLP and if so, was there a detectable interaction between these genes. Fourteen folate metabolism related genes were interrogated using 94 SNPs (single nucleotide polymorphisms) in 325 parent-child trios and 120 multiplex NSCLP families. These families were either of nonHispanic white or Hispanic ethnicity. Evidence for association between NSCLP and SNPs in the BHMT2 (betaine-homocysteine methyltransferase 2), MTHFD1 (methylenetetrahydrofolate dehydrogenase 1), TYMS (thymidylate synthetase), and CBS (cystathionine-beta-synthase) genes was detected in the nonHispanic white samples, whereas evidence for association with SNPs in the TYMS, CBS, and MTR (methionine synthase) genes was detected in the Hispanic samples. Over-transmitted SNP haplotypes were identified in many of the genes in both population samples. Gene-gene interactions were also found in both groups. These data suggest that there is no direct association with the common SNPs of the MTHFR gene and NSCLP, but that the C677T polymorphism may interact with SNPs from other genes in the folate pathway to create a risk for NSCLP.

Pathogenesis and treatment of retinal degeneration in the rd16 mouse: a model for syndromic disorders caused by mutations in the centrosomal-ciliary protein CEP290/NPHP6. *R. A. Rachel[1], R. Kandpal[1], J. Nellissery[1], Z. Wu[2], C. Murga-Zamalloa[3], L. L. Bell[1], R. Farris[4], B. Chang[5], H. Khanna[3], P. Colosi[2], A. Swaroop[1]* 1) Neurobiology, Neurodegeneratlon & Repair Laboratory, National Eye Institute, Bethesda, MD; 2) Ocular Gene Therapy Laboratory, National Eye Institute, Bethesda, MD; 3) University of Michigan, Ann Arbor, MI; 4) Biological Imaging Core, National Eye Institute, Bethesda, MD; 5) Jackson Laboratories, Bar Harbor, ME.

Diverse mutations in CEP290 cause a spectrum of ciliopathies, including Joubert syndrome, Meckel-Gruber syndrome, Bardet-Biedl syndrome and Leber congenital amaurosis (LCA). We have shown that an in-frame deletion in the myosin-tail (myo-tail) domain of CEP290 in the retinal degeneration 16 (rd16) mouse and potential hypomorphic mutations in LCA patients result in a restricted phenotype with defects in retinal, olfactory, and possibly other sensory neurons. To dissect the function of CEP290 and pathogenesis of associated disease(s), we are taking several approaches. First, we have identified proteins that potentially interact with CEP290 by immunoprecipitation and mass spectroscopy, and by yeast two-hybrid analysis. Secondly, we are evaluating the time course and pathology related to retinal degeneration in rd16 retina. Thirdly, we are evaluating the ability of an AAV5-Cep290 gene transfer vector to complement the ciliopathy in postnatal rd16 mice. Collectively, these studies should assist in understanding the mechanism of restricted neurodegeneration phenotype in patients with CEP290 mutations.

Kelvin: A 2nd generation software package for computation of the PPL framework. *Y. Huang[1], A. Segre[2], J. O'Connell[3], W. Valentine-Cooper[1], S. Seok[1], V. Vieland[1,4]* 1) Battelle ctr for Mathematical Medicine, The Res Inst at Nationwide Children's Hospital, Columbus, OH; 2) Computer Science Dept, U. of Iowa, Iowa City, IA; 3) U. of Maryland, College Park, MD; 4) Pediatrics Dept, Ohio State University, Columbus, OH.

KELVIN is a comprehensive statistical genetic analysis software package developed under the PPL framework. At its core is a 2nd generation distributed multiprocessor linkage and linkage disequilibrium analysis program. It currently supports more than 20 different types of analyses, including combinations of marker-to-marker or trait-to-marker analysis, dichotomous trait, quantitative trait or QT threshold analysis, two point or multipoint analyses based on sex averaged or sex specific maps, linkage and trait-marker linkage disequilibrium, and gene x gene interaction models. Kelvin uses Bayesian sequential updating to accumulate evidence across multiple data sets. It is designed for ease of expansion to allow new features in order to quickly address new research questions. Its supported platform base has been widened to include Macintosh OSX and Windows XP/Vista in addition to LINUX. Its performance both in terms of execution time and memory utilization has been radically improved such that it is now capable of performing many analyses that were formerly too complex to be computationally tractable. A few of the techniques employed in KELVIN include polynomial representation of likelihoods for fast evaluation, and an innovative and very efficient numerical integration method. A new graphical interface has been incorporated for setting-up analysis parameters in a user-friendly fashion. The package also provides an easy-to-use graphing tool for displaying analysis results. A new feature added to KELVIN is the ability to analyze case-control analyses for use in genome wide association studies, with the ability to simultaneously analyze case-control and family data utilizing sequential updating to accumulate evidence across potentially heterogeneous data sets or subsets.

The need for microarray testing in patients with presumptive diagnoses of mitochondrial disease. *C. Phornphutkul[1,2], D. Mandelbaum[1,2], R. Burke[1,2], K. Reddy[3], P. Cotter[3], S. Gunn[3], N. Shur[1,2]* 1) Hasbro Children's Hospital, Providence, RI; 2) The Warren Alpert Medical School of Brown University, Providence, RI; 3) Combimatrix Molecular Diagnostics, Irvine, CA.

Introduction: In patients with psychomotor retardation, seizures, and hypotonia, mitochondrial diagnosis is often suspected. Muscle biopsy has an integral role in the evaluation, though limitations are well-documented. We describe a case that highlights the need to include microarray for patients in this category. Case report: The patient presented to metabolic clinic for the first time at 9 ½ years with a long-standing history of mental retardation, psychomotor delay, possible seizures, and presumed metabolic disease. Birth history included club-feet, and ventricular septal defect. Prior work-up showed normal high resolution karyotype and FISH for velocardiofacial and Smith-Magenis syndromes. Testing for Rett and Angelman syndrome were negative. Metabolic work up included normal very long chain fatty acids, phytanic acids, congenital disorders of glycosylation, amino acids, and urine organic acids. Lactate and pyruvate and mitochondrial DNA studies were normal. A muscle biopsy showed a mildly elevated butcitrate synthetase level and mitochondrial proliferation. The presumptive diagnosis of mitochondrial disease was given to the family. Physical examination at 9 1/2 years was significant for synophorys, a prominent forehead, and a low anterior hairline with simple ears. Muscle tone was diffusely decreased with abnormal gait. Due to history of congenital heart disease and mild facial dysmorphism, microarray testing was obtained. A 105K oligoarray platform showed a gain of ~5.9 Mb of DNA (152059382 -158018753 bp) on chromosome 1q21.2 containing ~137 genes. High density BAC aCGH confirmed the diagnosis. The genes in the region had no apparent direct mitochondrial function or interaction with the mitochondrial genome. Conclusion: Our case highlights the need for microarray testing in patients with psychomotor delay, seizure and hypotonia, especially prior to invasive testing.

Craniosynostosis: Clinical insights and identification of a potentially new locus. *D. M. McDonald-McGinn[1], C. Stolle[2], T. Shaikh[1], C. Haldeman-Englert[1], S. Bartlett[3], E. H. Zackai[1]* 1) Division of Human Genetics; 2) Department of Pathology and Laboratory Medicine; 3) Division of Plastic and Reconstructive Surgery, The Children's Hospital of Philadelphia, Philadelphia, PA.

Classic syndromic craniosynostosis such as is seen in Crouzon, Pfeiffer, Jackson-Weiss, Muenke, and Saethre-Chotzen syndrome has been associated with mutations in FGFR 1, 2, 3 and TWIST and with TWIST deletions. However, there are a number of patients with apparent syndromic craniosynostosis whose etiology has yet to be elucidated. Here we report on two patients with craniosynostosis due to TWIST mutations whose clinical findings are dramatically divergent from each other. In addition we have identified a patient with craniosynostosis due to an interstitial 4q deletion and, having reviewed the literature, propose this as a potentially new craniosynostosis locus. Patient 1, identified intrauterine with bicoronal and metopic suture fusion necessitating emergent decompression after birth, was found to have an in frame duplication of seven amino acids (Lys133Pro139) in the loop domain region of the TWIST gene. Patient 2, a 32-year-old PhD student with bicoronal synostosis, blepharophimosis, and small ears, was found to have a complete deletion of the TWIST gene by southern blot, later confirmed by FISH. Patient 3, a 4 year old with posterior sagittal, lambdoidal and bicoronal craniosynostosis, hypertelorism, and sensorineural hearing loss was found to have a 4q27-q31.22 deletion detected using array CGH. A similar patient with craniosynostosis and an interstitial deletion of this same area was reported previously by DeValle Torrado et al. (J Med Genet 19:477) in 1982. Thus, we will discuss the genotype phenotype correlations in Patients 1 & 2 and propose the 4q27-q31.22 deletion as a potentially new craniosynostosis locus. High density oligonucleotide microarray identifying genes in this area will be discussed with attention to possible candidate genes.

Web-based collection of gene sequence variants and their phenotypic consequences. *I. F. A. C. Fokkema, P. E. M. Taschner, G. J. B. Van Ommen, J. T. Den Dunnen* Human & Clinical Genetics, Leiden Univ Medical Ctr, Leiden, Netherlands.

Soon it will be possible and affordable to sequence a human genome within a few weeks, making medical and clinical application of genome sequencing an attractive option. However, when we want to understand the consequences of all the variation we will find, we need to improve considerably the way we currently report and catalogue these variants and their consequences. One logical and diagnostically attractive option is to store this information on a gene-by-gene basis, i.e. in Locus-Specific DataBases (LSDBs). To facilitate fully web-based management of LSDBs we have developed the Leiden Open-source Variation Database software (LOVD, http://www.LOVD.nl). LOVD is a free, open-source, platform-independent tool to build, curate and share gene variant databases. After installation, simply from CD, a basic database is available following current recommendations of the Human Genome Variation Society. The database manager can add any data field desired, e.g. to capture disease-specific phenotype information, deciding per field the input accepted (free text, numeric, dropdown menu, etc.) and whether or not those data will be available for public display. LOVD supports several levels of data access (website visitor, submitter, curator, database manager), searching in (using Boolean operators) and across data columns, custom design of direct links (to internet, intranet or even local-PC files), data exchange with central repositories (incl. NCBI, UCSC), automatic mutation nomenclature error checking using Mutalyzer and data storage on variants in different genes found in one patient. LOVD allows searching of data in non-public records, returning the number of hits and the option to mail the curator or submitter asking for more information. With the support of the EU-funded Gen2Phen project, LOVD currently offers free support to establish and host gene variant databases. Currently, LOVD is used to curate >160 LSDBs world-wide, with the Leiden server hosting over 100 and data collected for ~48,000 variants from >27,000 patients contributed by >240 submitters, the largest series covering gene variants in relation to neuromuscular disorders.

A whole-genome association study of schizophrenia in 6,792 individuals. *S. Purcell[1,2], J. Stone[1,2], E. Scolnick[1,2], P. Sklar[1,2], International Schizophrenia Consortium* 1) CHGR, Massachusetts Gen Hosp, Boston, MA; 2) Stanley Center for Psychiatric Research, Broad Institute of Harvard and MIT.

The International Schizophrenia Consortium (ISC) was established to promote rapid progress towards the identification of genetic causes underlying schizophrenia. The ISC is comprised of investigators from the University of Aberdeen, Cardiff University, the University of Edinburgh, the Karolinska Institutet, Massachusetts General Hospital, the University of North Carolina-Chapel Hill, the Queensland Institute of Medical Research, the University of Southern California, the Stanley Center for Psychiatric Research at the Broad Institute of Harvard and MIT, Trinity College Dublin and University College London. To identify alleles that increase risk for schizophrenia, we completed a genome-wide association study using the Affymetrix Genome-Wide Human SNP 5.0 and 6.0 Arrays in 3,380 unrelated schizophrenic patients and 3,592 ancestrally matched controls. Our analysis of over 750,000 directly genotyped single nucleotide polymorphisms (SNPs), augmented by imputation of ungenotyped SNPs, revealed loci at 6q21 ($P = 2 \times 10\text{-}8$, rs2894254) and 1q21 ($P = 5\times10\text{-}8$, rs11165690) as significantly associated with schizophrenia. We have also completed a number of secondary analyses including tests of multilocus and pathway models and runs of homozygosity.

Triphalangeal thumbs in a patient with cat eye syndrome. *S. L. Dugan, L. Hudgins* Division of Medical Genetics, Stanford University School of Medicine, Stanford, CA.

Purpose: Cat eye syndrome is an uncommon form of aneuploidy consisting of a supernumerary isodicentric chromosome 22. Affected individuals present with multiple anomalies, including congenital heart disease, anorectal malformations, preauricular pits and tags, and ocular coloboma. We present a newborn with triphalangeal thumbs whose karyotype shows supernumerary isodicentric chromosome 22. This case expands the reported phenotypic range of cat eye syndrome and highlights its overlap with Townes-Brocks syndrome.

Methods: Chart review and a complete history and physical examination were performed on the patient. Karyotype was performed on peripheral blood. The patients father also underwent physical examination.

Results: The infant had downslanting palpebral fissures with telecanthus, bilateral radially deviated thumbs, bilateral preauricular pits and tags, and imperforate anus. Imaging studies revealed total anomalous pulmonary venous return, hydronephrosis, and triphalangeal thumbs. Karyotype was 47,XY,+idic(22)(q11.2) in all 20 cells examined. The patients cognitively normal father has a unilateral triphalangeal thumb and bilateral preauricular pits.

Conclusions: As triphalangeal thumb has not previously been described in an individual with cat eye syndrome, this case expands the reported phenotypic spectrum of the condition. The finding of triphalangeal thumbs in our patient calls further attention to the clinical overlap between cat eye syndrome and Townes-Brocks syndrome, an autosomal dominant condition resulting from alteration of the *SALL1* gene. Ongoing elucidation of the molecular mechanisms behind the Townes-Brocks phenotype and mapping of the cat eye syndrome critical region will suggest investigative pathways into the molecular mechanisms behind the cat eye syndrome phenotype.

Novel variants identified in methyl-CpG-binding protein genes. *H. N. Cukier[1], R. Rabionet[1], I. Konidari[1], M. Y. Rayner[1], M. L. Baltos[1], H. H. Wright[2], R. K. Abramson[2], M. L. Cuccaro[1], M. A. Pericak-Vance[1], J. R. Gilbert[1]* 1) Miami Institute of Human Genomics, University of Miami, Miami, FL; 2) University of South Carolina School of Medicine, Columbia, SC.

Misregulation of the *methyl-CpG-binding protein 2* (*MECP2*) gene causes a myriad of neurodevelopmental disorders characterized by social and intellectual impairmentsmost notably Rett syndrome and some cases of autism. We hypothesized that mutations in additional members of the methyl-CpG-binding family (*MBD1, MBD2, MBD3* and *MBD4*) may also be involved in autistic spectrum disorders (ASD). This is further supported by evidence that different MBD proteins are capable of binding the same promoter region, suggesting a functional interdependence. To date, only one study has evaluated the MBD genes of autistic patients and was limited to a Japanese population. A single variation of interest was identified, R269C in MBD1. In this study, 230 autistic individuals (198 Caucasians (CA) and 32 African-Americans (AA)) were evaluated for the coding regions of *MBD1-4* by denaturing high performance liquid chromatography and results were confirmed either by TaqMan or direct sequencing. We used both multiplex and singleton families and examined transmission and disease concordance. We identified 91 autistic individuals (71 CA and 20 AA) carrying genetic alterations at 50 unique loci. 19/50 were known single nucleotide polymorphisms (SNPs), while 31/50 were novel (6 insertion/deletions and 25 SNPs). While the majority of alterations were synonymous or noncoding SNPs, we identified 9 non-synonymous changes in MBD1, 3 and 4, the deletion of a single amino acid in MBD3, and a frameshift mutation in MBD4 that truncates almost half of the protein. We did not find the MBD1 R296C variant. Ten of the novel SNPs identified in the CA affecteds, 9 of which were inherited, were not found in 300 CA control alleles. Similar studies are ongoing in AA controls. The investigation of these rare variants indicates a potential role for the MBD genes in the molecular and genetic etiology of autism, thereby furthering our understanding of this complex disorder.

Identification of a novel *DSPP* mutation which shows that the Dentinogenesis Imperfecta vs. Dentin Dysplasia phenotypes are due to a linked genetic modifier and not the location of the mutation within exon 5. *D. A. McKnight[1], P. S. Hart[2], T. C. Hart[1], L. W. Fisher[1]* 1) CSDB, NIDCR/NIH, Bethesda, MD; 2) Office of the Director, NHGRI/NIH, Bethesda, MD.

Dentinogenesis Imperfecta types II and III (DGI) and Dentin Dysplasia type II (DD) are the major, nonsyndromic autosomal dominant diseases of dentin. DGI affects both the primary and permanent dentition while only the primary teeth are severely affected in DD. While both diseases have been linked to a single gene, dentin sialophosphoprotein (*DSPP*), the clinical diagnosis of each affected member of extended families remain the same. Recently, we described a new series of frameshift mutations in the 2 kb repetitive portion of DSPPs exon 5 that is predicted to change the long hydrophilic tandem repeat (~220 x SerSerAsp) into 100-600 amino acid hydrophobic domains rich in Ile, Val and Ala. This long stretch of hydrophobic residues will most likely cause the translated proteins to precipitate in the rER and therefore interfere with odontoblast processing of normal DSPP and/or other matrix proteins such as type I collagen. This hypothesis also explains the dominant phenotype in these disorders. Based on our results and those published simultaneously by another laboratory, all reported DD frameshifts were 5 to all DGI mutations. Here we describe a novel frameshift mutation (c.3139delC) from an extended DD family that is found within the proposed DGI-mutation domain. This newly identified mutation therefore indicates that the length of mutant hydrophobic protein itself does not determine the disease outcome of the DGI vs. DD phenotypes. Instead, we propose the strictly inherited phenotypes within affected families are the result of a yet-to-be-identified genetic modifier closely linked to the *DSPP* gene, perhaps within the promoter or mRNA-stabilizing domain within the 3 UTR. This research was supported [in part] by the Intramural Research Program of the NIH, NIDCR.

Prenatal diagnosis of chromosomal abnormalities by clinical array comparative genomic hybridization (aCGH).

I. Van den Veyver[1,2], S. Kang[2], C. Shaw[2], A. Pursley[2], P. Ward[2], S. Neill[2], W. Bi[2], A. Breman[2], P. Eng[2], C. Eng[2], L. White[2], J. Lupski[2], S. Cheung[2], A. Beaudet[2] 1) Dept of Ob-Gyn; 2) Dept of Molec Human Genetics, Baylor Coll Medicine, Houston, TX.

Experience with aCGH for prenatal diagnosis is still limited. We evaluated the performance of a targeted clinical aCGH test for prenatal diagnosis of unbalanced chromosomal abnormalities. Women who underwent amniocentesis or chorionic villus sampling (CVS) were offered aCGH as an adjunct test on a fee basis. Fetal DNA was analyzed on targeted BAC or oligonucleotide arrays, optimized for detection of genomic disorders with reduced detection of polymorphic copy number variants (CNV). All samples were also cultured for karyotype analysis or needed confirmatory studies. Parental samples were obtained concurrently to determine if detected CNVs were inherited. We analyzed 350 samples: 217 amniotic fluid (AF) (62%), 38 CVS (11%), 70 already established amniocyte cultures (21%), 23 chorionic villi cultures (6%), 1 cystic hygroma fluid and 1 fetal skin biopsy. Results were reported on DNA directly extracted from uncultured samples in 113/217 AF (52%), of which 56/113 (50%) required prior whole genome amplification (WGA), and in 32/38 (84%) CVS, of which 5/32 (16%) required prior WGA. No gain or loss was detected in 285/350 samples (81%). There were 65/350 CNVs, 41/350 (12%) of which were interpreted as likely benign, 20/350 (6%) of defined clinical significance and 4/350 (1%) of uncertain clinical significance, the most difficult of which was a de novo deletion 16p13.1 (PMID: 17480035). For 11/350 samples (~3.1% or 1/32), aCGH contributed important new information. For 4/350 (1 % or ~1/88), the abnormality would not have been detected without aCGH analysis. Array CGH also facilitated rapid identification of 5 of 10 supernumerary marker chromosomes found on the fetal karyotype. We conclude that aCGH is an important new diagnostic tool for prenatal diagnosis of submicroscopic and larger unbalanced genomic rearrangements and for identification of the genomic content of marker chromosomes. Although aCGH detected benign inherited variants in 12% of cases, these did not present major counseling difficulties.

Identification of modifier genes for cutaneous malignant melanoma (CMM) and dysplastic nevi (DN) in melanoma families with and without CDKN2A mutations. *R. Yang, R. Pfeiffer, M. Tucker, A. Goldstein, Core Genotyping Facility* DCEG/NCI/NIH/DHHS, Bethesda, MD.

The CDKN2A gene is the major high-risk melanoma susceptibility gene identified to date. The variable penetrance and clinical manifestations among mutation carriers suggest the existence of modifier genes or other risk factors. The presence of dysplastic nevi (DN) is also a strong risk factor for familial CMM and to date no candidate genes have been identified for DN. The goal of this study is to identify low-penetrant genes for CMM and DN in CMM/DN-prone families with and without CDKN2A mutations. We genotyped 537 individuals (107 CMM, 209 DN, 135 CDKN2A mutation carriers) from 28 CMM families (19 CDKN2A+, 9 CDKN2A-) for 1536 SNPs in 152 genes included in a custom designed Illumina panel, which includes genes involved in important pathways such as DNA repair, apoptosis, and immune responses. We used conditional logistic regression conditioning on families to detect SNPs that are associated with the risk of CMM and DN as separate outcomes. As expected, we found that some genes had differential effects for CMM or DN, whereas some were associated with both outcomes. Most notably, SNPs in BCL2 (OR_{het} = 0.4 [0.25, 0.65]; OR_{homo} = 0.27 [0.13, 0.54]; P_{trend} = 0.0003) and IL10RB (OR_{het} = 1.91 [1.22, 2.99]; OR_{homo} = 2.71 [1.28, 5.76]; P_{trend} < 0.0001) were only associated with the risk of CMM; whereas SNPs in TNFSF13B had stronger effects on DN (OR_{het} = 0.43 [0.26, 0.71]; OR_{homo} = 0.18 [0.02, 1.56]; P_{trend} = 0.0004). Genes such as TNFRSF8 and IL1R1 were associated with increased risk of both CMM and DN. Risk estimates were similar among CDKN2A carriers and non-carriers for most of these SNPs. We also used a random effect model (Pfeiffer R, Biometrika, 2001) to further account for residual familial correlations among family members and the effect estimates obtained from the random effect model were more significant compared to those from logistic regression analysis. In the future, we will examine specific gene and pathway effects and will also incorporate host and sun exposure variables in the model. In addition, we will validate these findings in larger, independent datasets.

Genetic variants at 17q21 locus contribute to early-onset asthma and interact with environmental tobacco smoke exposure in early-life. *E. Bouzigon[1], E. Corda[1], H. Aschard[1], MH. Dizier[2], N. Chateigner[1], I. Pin[3], F. Kauffmann[4], M. Lathrop[5], F. Demenais[1]* 1) U794, INSERM, CEPH, Paris, France; 2) U535, INSERM, Paris, France; 3) U823, INSERM, Grenoble, France; 4) U780, INSERM, Villejuif, France; 5) CNG, Institut de Génomique, CEA, Evry, France.

A genome-wide association (GWA) study identified genetic variants at chromosome 17q21 that modulate expression of *ORMDL3* and are associated with an increased risk of asthma. To elucidate further the relationship of this locus with disease, we investigated whether the effects of these variants differ according to age-of-onset of asthma and interact with environmental tobacco smoke (ETS) exposure in early-life. We tested 36 SNPs in 17q21 region in 1,511 subjects from 372 French EGEA families. Association analysis was carried out using a likelihood-based method implemented in LAMP program. We first replicated association of asthma with 11 SNPs (P<0.01) of which 3 obtained P<0.001. We then conducted ordered-subset regression analysis that led us to select onset at or before 4 years of age to classify patients into early-onset and late-onset asthma. Association with early-onset asthma was found to be highly significant ($P<10^{-5}$ for 4 SNPs), while no association was found with late-onset asthma (P0.002 against homogeneity). When examining ETS exposure in early-life, association with early-onset asthma was only significant in the exposed families ($P<5x10^{-5}$ for 6 SNPs). The early-onset asthma odds-ratios (ORs) were consistently increased by ETS exposure: OR=3.11 (95% confidence interval, 1.79 to 5.42, under the best fitting recesive model)for rs8069176, the most strongly associated SNP. Furthermore, we showed that a single haplotype block accounts for association with early-onset asthma, thus narrowing down the region of interest as compared to the initial GWA study. This study demonstrates that increased risk conferred by 17q21 SNPs is restricted to early-onset asthma and is further enhanced by ETS exposure in early-life. These results are an important contribution to the understanding of the mechanisms by which 17q21 genetic variants act and have important implication for improving prevention strategies against asthma.

Association Analysis of Serotonin and Dopamine Transporter Genes with Posttraumatic Stress Disorder (PTSD).

H. Munger[1,3], R. Morey[2,3], J. Rimmler[1,3], M. F. Dennis[2,3], K. Green[2,3], M. A. Hauser[1,3], S. Schmidt[1,3], J. C. Beckham[2,3] 1) Center for Human Genetics, Department of Medicine, Duke University Medical Center, Durham, NC; 2) Department of Psychiatry and Behavioral Sciences, Duke University Medical Center, Durham, NC; 3) Mid-Atlantic Mental Illness Research, Education and Clinical Center (MIRECC), Durham VA Medical Center, Durham, NC.

The serotonin (SLC6A4) and dopamine (SLC6A3) transporter genes have previously been investigated as candidate genes for several psychiatric disorders, including PTSD. As part of the Mid-Atlantic MIRECC, we have psychiatrically evaluated over 900 individuals, most of whom are veterans returning from recent deployment to Iraq or Afghanistan. To date, 572 DNA samples have been genotyped for a total of 27 tagging SNPs in SLCA3 and SLCA4, and for two functional polymorphisms: the 5HTTLPR/rs25531 promoter variant in SLCA4 and the 3 VNTR in SLCA3. Caucasian subjects carrying either the low- or high-expressing genotypes at 5HTTLPR had an increased risk of lifetime PTSD, relative to individuals with normal SLCA4 expression levels: OR 2.34, 95% CI 0.99-5.53 for high expression; OR 2.42, 95% CI 1.09-5.37 for low expression (72 cases/78 controls). The effect size increased (to OR 2.83, 95% CI 1.15-7.0 for high expression) when we adjusted for level of lifetime trauma exposure. Genotypes at the intronic SNP rs420422 in SLC6A3 were associated with self-reported current PTSD in both Caucasians and African-Americans (uncorrected p=0.005), regardless of the extent of traumatic events. In addition, a significant association with any Axis 1 disorder was detected for the intronic SNP rs250682 in SLC6A3 in Caucasians (p=0.008) and for the intronic SNP rs9303628 in SLC6A4 in African-Americans (p=0.005). These results suggest that SLC6A4 and SLC6A3 genotypes are associated with PTSD specifically and psychiatric disorders more generally, and that these associations vary across ethnicities. Furthermore, they suggest that lifetime trauma exposure may modulate the 5HTTLPR association with PTSD.

Inherited and de novo copy number variation changes in neurodevelopmental disorders. *S. Villatoro[1], L. Armengol[1], I. Crespo[4], E. Gabau[2], L. Comadran[4], MD. Coll[3], M. Guitart[4], X. Estivill[1,5]* 1) Genes and Disease, Center for Genomic Regulation; 2) Servei de Pediatria, Corporació Sanitària Parc Taulí; 3) Departament de Biologia Cellular Universitat Autònoma de Barcelona; 4) Laboratori de Genètica, Corporació Sanitària Parc Taulí; 5) Pompeu Fabra University, Barcelona, Spain.

Angelman syndrome (AS) is a neurodevelopmental disorder with a recognizable molecular cause in about 85% of cases. Copy number variation (CNV) is an important source of genomic changes, and genes located therein are likely to exhibit altered expression patterns, therefore contributing to phenotypic changes. Twenty AS patients without the typical molecular alterations but with well-defined clinical features were analyzed by aCGH using the 244K Agilent platform. Altered regions that contained rare CNVs (1 finding in the literature) or not reported in the Database of Genomic Variants were selected for validation using custom Multiplex Ligation-dependent Probe Amplification (MLPA) assays (83 probes targeting 73 different regions). We assessed the CNV status in the 20 AS cases and in their parents, and also expanded the study to larger sets of samples of individuals suffering idiopathic mental retardation (n=296), autism (n=164) as well as to a control cohort of normal individuals (n=453). We identified 3 de novo deletions (1p36, 1q44 and ZNF528 gene), two maternally inherited duplications (Xp11.23 and Xq28), 19 inherited altered regions present in disease cases but not identified in our control population. We also identified 37 inherited genomic variants that are present in the general population. Three of those exhibit significant frequency differences between cases and controls ($P<0.05$; Fisher Test). Our results support the view that a considerable proportion of genomic regions showing variability in copy number could be involved in neurodevelopmental disorders. The absence of genomic abnormalities in controls of inherited genomic changes detected in cases, suggests that even if inherited, they could be responsible for some of the clinical features perhaps unmasking recessive mutations in specific genes involved in the phenotypes.

***IL18* and *IL2* polymorphism in Psoriasis susceptibility in the Irish population.** *A. Ryan[1], K. Al-Jubury[1], G. Turner[1], P. Gallagher[2], A. Irvine[1], O. Fitzgerald[2], B. Kirby[2], R. McManus[1]* 1) Department of Clinical Medicine, Institute of Molecular Medicine, Trinity College, Dublin, Ireland; 2) St Vincents University Hospital, University College, Dublin, Ireland.

Psoriasis is an inflammatory disease of the skin and joints with a prevalence of up to 2% in European populations. IL18 is a pro-inflammatory cytokine, which promotes Th1 T-cell development by inducing -interferon. Genetic variation in this gene has been implicated in the pathogenesis of several autoimmune conditions, including inflammatory bowel disease, type 1 diabetes, atopic eczema and asthma. There is some evidence that IL18 may play a role in psoriasis. rs6840968 is a single nucleotide polymorphism (SNP) within the *IL2/IL21* region of chromosome 4q27, which has recently been associated with Type I Diabetes, Rheumatoid Arthritis, Graves Disease and Celiac Disease. The functional relevance of the *IL2* and *IL21* genes in inflammatory and autoimmune conditions is highlighted by the role of these cytokines in T-cell activation and development. We have genotyped *IL18* rs187238 (*IL18-137*) and *IL2/IL21* rs6840968 in 231 ethnically uniform Irish patients with psoriasis and 871 Irish controls. Both SNPs conformed to Hardy Weinberg Equilibrium in both populations. Both loci showed evidence of association with psoriasis in this population (*IL18* rs187238, Odds Ratio 0.61 [0.44 - 0.86], ^{2} = 8.99, P = 0.0027; *IL2/IL21* rs6840978, Odds Ratio 0.67 [0.48 - 0.92], ^{2} = 6.41, P = 0.01134, for carrier status of the minor allele in both cases). This latter value for *IL2/IL21* rs6840978 is similar to a recent study of psoriasis susceptibility for the same SNP in UK and US populations (OR = 0.77 [0.62 - 0.95] and OR = 0.81 [0.68 - 0.96], respectively), for the same allele and direction, substantiating existing evidence that the *IL2/IL21* haplotype block represents a risk factor for the development of psoriasis.

An education strategy for addressing barriers to the entry of genomic medicine in community healthcare. *K. Powell[1], C. Christianson[1], S. Hahn[2], S. Blanton[2], M. Pericak-Vance[2], V. Henrich[1]* 1) Univ NC at Greensboro, NC; 2) Univ of Miami, FL.

The Genomedical Connection is a demonstration project to develop and test a model for the integration of genomic medicine into primary care. The project includes an education program that addresses a variety of barriers within three stakeholder populations: the community, the healthcare providers, and their patients. Barriers for each audience were identified through a front end needs assessment utilizing surveys and focus groups. Identified barriers for the community and patients included a lack of knowledge about genetic concepts, terms and information about the health of other family members, low literacy and low health literacy. Provider barriers identified include the lack of: knowledge about genetics, genetic testing and/or genetic counseling, confidence in handling patient concerns raised in a family history, time to collect, assess and address family history, supportive resources, and protocols for managing patient healthcare after initial risk assessment. All audiences were concerned about privacy and confidentiality. To address these barriers we developed a comprehensive education strategy with coordinated and complementary educational objectives for each audience. Educational materials and events were developed in collaboration with community-based health professional organizations, a community advisory board, and partner organizations. Community and patient education materials and presentations were developed at a 6th-8th grade level. To address a subset of the provider barriers, on-line education modules offering CME credit for family history and 3 pilot diseases (breast/ovarian cancer, colon cancer, and thrombophilia) were created to teach providers how to recognize patients who may be at risk based on family health history, to stratify them into risk categories, and to manage their care accordingly. Our research indicates that the successful integration of genomic medicine into the primary care setting will require that numerous barriers be addressed; targeted education is the first of a multifaceted effort to be implemented.

Direct access genetic tests advertised online. *A. Roche*[1,2], *D. Doyle*[1,2] 1) Washington State Department of Health, Kent, WA; 2) Genetic Services Policy Project, Seattle, WA.

The number of genetic tests offered directly to the public via the internet is growing, raising concerns as well as providing the potential for benefit. Using a list of 31 companies tracked by the Genetics and Public Policy Center, we reviewed websites advertising direct access genetic tests to characterize the types of tests offered, marketing approaches employed, the accuracy of information provided, and the extent of genetic counselor or other health care provider involvement in the testing process. We found wide variation in all of these areas. Tests offered include whole genome sequencing, single nucleotide polymorphism (SNP) genome profiles, SNP panels for complex disease risk or to create a personalized intervention product, and single-gene Mendelian disorders, with prices ranging from $75 to $350,000. Five companies provide access to a genetic counselor as part of the test purchase price, but the majority do not address genetic counseling at all. When provided with appropriate support, some online genetic tests may help improve consumer access. To protect consumers from harms that might arise in online genetic services markets, the Advisors to the Genetic Services Policy Project, a federally funded grant to assess genetic services delivery and translation of new research into practice, recommend several policy actions. The federal government may: a) consider regulating home brew tests, b) evaluate the need for broad regulations regarding direct access genetic tests, c) assure that the Federal Trade Commission, Food and Drug Administration, and state attorneys general carry out their mission of preventing harm and fraud, and d) consider licensing companies selling direct access genetic tests.

Natural history and phenotype of NF1-associated glomus tumors. *J. Sloan[1], A. Moshyedi[2], L. Yao[2], R. Lee[2], D. Stewart[1]* 1) NHGRI, Bethesda, MD; 2) NIH Clinical Center, Bethesda, MD.

Glomus tumors are benign tumors that arise from the glomus body, a thermoregulatory shunt in the fingertips. Multi-focal glomus tumors in patients with neurofibromatosis type 1 (NF1) have been reported suggesting a possible association. Seven adults with NF1 recruited for a natural history study reported classic glomus tumor symptoms, were evaluated by MRI and in 4 cases surgery was performed. Patient 1 was a 35-year-old female with a 5-year history of pain in 6 digits. MRI revealed lesions in 3/6 symptomatic digits. Two surgeries were performed over a 6-month period. Three lesions were histologically confirmed glomus tumors. The other 3 digits showed enlarged Pacinian corpuscles. Between the 1st and 2nd surgeries, a glomus tumor recurred in the left 4th digit. The pain returned and a 3rd explorative surgery was conducted in 3 fingers. No glomus tumors were identified. Six months after the 3rd surgery the pain returned. Patient 2 was a 50-year-old male with a 15-year history of pain and disuse of his left arm with symptoms consistent with complex regional pain syndrome (CRPS). In 3 fingers, glomus tumors were identified by MRI and removed. Six months later the pain returned and surgery confirmed recurrence in all 3 digits. Six months after the 2nd surgery the pain returned. Patient 3 was a 28-year-old female with a 4-year history of pain in 3 fingers. MRI identified a 3 mm lesion in the left 3rd digit only. Surgical exploration revealed a glomus tumor in this digit but none were identified in the other painful fingers. Eight months later pain in the left 3rd digit returned. Patient 4 was a 48 year-old female with pain in the left 3rd digit since childhood. A 3 mm lesion was identified on MRI, surgery was performed and revealed a glomus tumor. Six weeks postoperatively the pain resolved. Our experience supports an association between NF1 and glomus tumors. Glomus tumor symptoms were variable ranging from intermittent pain to CRPS. Two patients had recurrence of their glomus tumors in less than a year. Early intervention may be desirable to prevent the development of CRPS. Clinicians should routinely ask about fingertip pain in patients with NF1.

The rs1333049 SNP near CDKN2a/2b, associated with Myocardial Infarction, is associated with increased risk of Peripheral Artery Disease in older people. *C. Cluett[1], T. Tanaka[2], A. Kisialiou[3], J. Guralnik[4], L. Ferrucci[2], T. M. Frayling[5], A. Murray[1,5], D. Melzer[1,5]* 1) Epidemiology and Public Health, Peninsula Medical School, Exeter, Devon, United Kingdom; 2) Longitudinal Studies Section, Clinical Research Branch, Gerontology Research Center, National Institute on Aging, Baltimore, Maryland, United States of America; 3) Tuscany Regional Health Agency, I.O.T. and Department of Medical and Surgical Critical Care, University of Florence, Florence, Italy; 4) Department of Geriatric Medicine Laboratory of Epidemiology, Demography and Biometry, National Institute on Aging, Bethesda, Maryland, United States of America; 5) Genetics of Complex traits, Institute of Biomedical and Clinical Sciences, Peninsula College of Medicine and Dentistry, University of Exeter, UK.

Recent human genome wide association studies have found consistent associations between a common variant near the CDKN2a/2b loci on chromosome 9p21 and Myocardial Infarction (MI) /Coronary Artery Disease (CAD). Proximal to this polymorphism lie two cyclin dependent kinase inhibitor genes, CDKN2a and CDKN2b, which are are key regulators of the cell cycle. The variant has also been shown to be associated with abdominal aortic and intracranial aneurysms, independent of the validated effect on CAD. However, similar associations with peripheral artery disease (PAD) and cardiogenic stroke were not significant when subjects with known CAD were excluded. We aimed to estimate the association between this variant (tagged by rs1333049) and Ankle Brachial Index and PAD in elderly people. In 2630 elderly individuals (mean age 76.4 years) the C allele of rs1333049 was associated with lower mean ABI measures (-0.017 ABI units, 95% CI: -0.03--0.01, p=1.3x10-4) and an increased risk of PAD (OR: 1.29 95% CI: 1.06-1.56, p=0.012) after removal of baseline and incident MI cases in a 6 year period. This is the first report of a common variant associated with MI and aneurysms, being independently associated with ABI and PAD.

SUMF1-activated IDS activity retained after fusion with a brain-targeting ligand: application in encapsulated cellular therapy. *M. J. Schwindt, M. A. Potter* Medical Sciences, McMaster University, Hamilton, ON, Canada.

Mucopolysaccharidosis type II (MPS II), also known as Hunter syndrome, is a rare X-linked genetic disorder which is characterized by the deficiency of the lysosomal enzyme iduronate-2-sulfatase (IDS). This deficiency results in lysosomal storage of the glycosaminoglycans (GAGs) dermatan sulfate and heparan sulfate, which in severe forms of the disease includes accumulation in the CNS, leading to developmental regression and shortened lifespan. The recent development of enzyme replacement therapy has achieved success in improving the somatic conditions of MPS II, however little to no GAG clearance occurs in the brain as the blood-brain barrier (BBB) impedes vascular delivery of the enzyme. In this study, mouse embryonic fibroblasts (MEFs) and C2C12 murine myoblasts have been co-transfected with modified IDS and its activator, sulfatase-modifying factor 1 (SUMF1). IDS was fused to the receptor-binding domain of Apolipoprotein B (ApoB), which has been used as a ligand to transport therapeutic molecules across the BBB via low-density lipoprotein (LDL) receptors, and tagged with an AU1 epitope for tracking purposes. IDS modification, including c- and n-terminal AU1 tags, ApoB fusions, and a combination of both, does not diminish enzyme activity secreted from cells in vitro, although cell type had a large effect on secretion (C2C12s had 5-fold more secretion than MEFs). Furthermore, co-transfection with SUMF1 leads to a two-fold increase in secreted enzyme activity compared to cells transfected with IDS alone. Immunofluorescence and Western blots, performed on the various constructs utilizing AU1-targeted antibodies, confirms the identity and intracellular localization of the constructs. Correction of human MPS II fibroblasts has been achieved following 48 h incubations in various conditioned media and in co-culture with microencapsulated engineered cells; thus indicating that the modified IDS is taken up from the medium and targeted to lysosomes. Future in vivo work will evaluate the efficacy with which ApoB is able to transcytose the BBB and deliver therapeutic IDS to MPS II mice.

Clinical and functional studies in subjects carrying glucocerebrosidase (GBA) mutations. *J. Davis[1], K. Berman[2], G. Lopez[3], TL. Urban[1,4], E. Sidransky[1], O. Goker-Alpan[1]* 1) MGB/NHGRI/NIH, Bethesda, MD; 2) NIMH/NIH, Bethesda, MD; 3) NINDS/NIH, Bethesda, MD; 4) UPENN School of Nursing, PA.

Multiple studies indicate that GBA mutations are a risk factor for parkinsonism. A prospective study was designed and initiated to identify and objectively characterize the early parkinsonian manifestations and the rate of progression of symptoms in affected and at risk individuals carrying GBA mutations. All subjects undergo neurological, neurocognitive evaluations and olfactory testing. Presynaptic dopaminergic function and cerebral blood flow are assessed with F-18-L-DOPA and 15 O-H2O PET scans. Transcranial ultrasonography is explored as a noninvasive tool to evaluate hyperechogenicity of the substantia nigra. The subjects include patients with Gaucher disease and Gaucher carriers with parkinsonism, and/or with a family history of a first degree relative with parkinsonism. The genotypes identified are N370S, L444P, c.84dupG and recombinant alleles. Among 21 subjects recruited to the study, there are 10 patients with parkinsonism and two discordant sib-pairs with Gaucher disease where only one has parkinsonism. In subjects with parkinsonism(7M: 3F), the mean age of onset was 49 years, disease duration was 8 years, and UPDRS III score was 26. Six subjects were given the diagnosis of classic Parkinson disease (PD). Half of the patients reported cognitive changes later in their disease course. Three subjects were considered to have Lewy body dementia (LBD), and one parkinson plus syndrome. The most frequent non-motor finding was olfactory dysfunction. Thus, GBA mutations, in both homozygotes and heterozygotes, are associated with a spectrum of parkinsonian phenotypes ranging from classic PD, to a less common phenotype characteristic of LBD. The results of the clinical and functional imaging studies will enable us to estimate the frequency and earliest onset symptoms in at-risk subjects, better define the associated parkinsonian phenotype, follow the progression of manifestations and identify at-risk individuals. This study will also help us to identify whether specific abnormalities in L-Dopa metabolism occur in subjects with GBA mutations.

Barth syndrome caused by a novel deletion mutation in the TAZ gene in a Hispanic kindred. *Y. Fan[1], R. Chang[2], R. L. Forst[2], L. S. Pena[1], A. C. McCanta[3], M. Vatta[1], J. A. Towbin[1]* 1) John Welsh Cardiovascular Diagnostic Laboratory, Department of Pediatrics (Cardiology), Texas Children's Hospital, Baylor College of Medicine, Houston, TX; 2) Division of Metabolic Disorders, Childrens Hospital Orange County, Orange County, CA; 3) Pediatric Cardiology, The Children's Hospital Denver, Aurora, CO.

Barth syndrome is an X-linked recessive disorder characterized by dilated cardiomyopathy, variably expressed skeletal myopathy, short stature, neutropenia, abnormal mitochondria, and 3-methylglutaconic aciduria. The disorder is caused by mutations in the tafazzin (TAZ/G4.5) gene located on Xq28, as first described by Bione et. al. in 1996. Alternative splicing of the gene yields several different proteins, the functions of which remain largely uncharacterized. Sequencing of the TAZ gene is currently the most reliable diagnostic approach for Barth syndrome. We describe a Hispanic family in which the 3-year-old proband presented with cyclic neutropenia, gross motor regression, decreased stature, dilated cardiomyopathy, and 3-methylglutaconic aciduria. His mother had two siblings, at least one of whom was male, who died in infancy of unknown causes. She had no other siblings. A novel 10-bp deletion mutation (728_737del10) in exon 10 of the TAZ gene was identified in the proband through bi-directional sequencing. This deletion creates a premature stop 20 codons downstream. Family studies revealed that the probands mother carries the same deletion, however, his maternal grandmother does not. The identification of TAZ gene mutations is important for early diagnosis of atypical Barth syndrome and the provision of accurate genetic counseling and carrier testing for at-risk relatives.

Impact of *APOBEC3B* gene deletion on HIV-1 infection and disease progression. *P. An[1], J. Phair[2], G. D. Kirk[3], S. Donfield[4], S. Buchbinder[5], J. J. Goedert[6], C. A. Winkler[1]* 1) Lab Genomic Diversity, SAIC-Frederick Inc, National Cancer Institute-Frederick, Frederick, MD; 2) Northwestern University, Chicago, IL; 3) Department of Epidemiology, Johns Hopkins School of Public Health, Baltimore, MD; 4) Rho, Inc., Chapel Hill, NC; 5) San Francisco Department of Public Health, San Francisco, CA; 6) Epidemiology Branch, National Cancer Institute, Bethesda, MD.

The human APOBEC3 family proteins are cytidine deaminases that inhibit virus, replication by inducing G to A hypermutation in the minus DNA strand during reverse transcription.. APOBEC3B, one of seven members of APOBEC3 family, has been shown to weakly or strongly inhibit HIV-1 replication *in vitro*. Recently, a common 29.5-kb deletion that removes the APOBEC3B gene was identified. We examined the impact of the *APOBEC3B* gene deletion on HIV-1 infection and disease progression in five well-characterized U.S.-based HIV-1 natural cohorts consisting of more than 4000 HIV-1 infected and exposed but uninfected subjects. The gene deletion was more frequent in European Americans (7.5%) than African Americans (4%). The analysis was done by a logistic regression for the comparison of HIV-1 infected and uninfected groups and by Cox proportional hazards model for the rate of AIDS progression stratified by the deletion genotype. The hemizygous deletion had no effect on either infection or progression in both populations. However, the homozygous deletion was significantly associated with increased susceptibility to infection and accelerated rate of progression to AIDS in European Americans. It is possible that other APOBEC3 family proteins can compensate for loss of one copy but not two copies of *APOBEC3B*. This finding suggests a role of *APOBEC3B* gene in HIV-1 pathogenesis. [Funded by NCI contract NO1-CO-12400].

Genome-Wide Copy Number Analysis in a Cohort 525 Probands with Non-Syndromic Bilateral Sensorineural Hearing Loss Using a Novel Analysis Software (PECONPI). *D. Clark[1], M. Berman[1], L. Conlin[1], H. Rehm[2], M. Kaur[1], J. Glessner[1,3], H. Hakonarson[1,3], S. Grant[1,3], N. B. Spinner[1,4], I. D. Krantz[1]* 1) Division of Human Genetics, The Children's Hospital of Philadelphia, PA; 2) Harvard/Boston Children's Hospital; 3) The Center for Applied Genomics, The Children's Hospital of Philadelphia, PA; 4) Division of Clinical Labs, Department of Pathology, The Children's Hospital of Philadelphia, PA.

SNP array platforms have been increasingly used to identify structural duplications and deletions (copy number variation or CNV) in patients with syndromic findings. Using these platforms for novel gene discovery in patients with single gene disorders has been hampered by the prevalence of small benign CNV in the genome. We have developed Perl Copy Numbers of Potential Interest (PECONPI), a program that ranks CNVs based on their pathogenic potential, scoring calls based on an adaptable set of parameters. Hearing loss is an ideal disorder to study using this approach as it is one of the most common hereditary human disorders, is highly heterogeneous, and most forms are believed to be caused by mutations in dominantly or recessively acting genes. Although there have been major breakthroughs in understanding the molecular basis of hearing loss, the causative genes in at least 60% of affected probands remain unidentified. We utilized the Illumina 550K SNP array to detect CNVs in 525 probands with non-syndromic sensorineural hearing loss (NSSNHL) and applied this novel software program to rank the CNV calls based on likely pathogenicity. By differentiating rare pathogenic CNVs from benign CNVs in this population, PECONPI was able to validate all known *GJB6* gene deletions in our cohort, identify novel deletion mechanisms in known hearing loss genes (e.g. *OTOF* and *USH2A*), and identify multiple novel candidate genes mapping to established and novel loci. PECONPI is a useful tool to identify new mechanisms and novel genes implicated in genetic hearing loss. It can be adapted for any population, and will prove useful for investigating disorders suspected to be caused by small genomic alterations, even those involving single genes.

Maternal-Grandpaternal Age Is a Potential Risk Factor for the Development of Autism Spectrum Disorder. *E. Faulks, O. Abdul-Rahman* Peds-Genetics, U of MS Med Center, MS.

Autism spectrum disorder and pervasive developmental disorders (ASD/PDD) usually present before the age of three years with impairments in communication and social interaction. Studies have provided strong evidence that ASD/PDD is a genetically heterogeneous entity. A few studies have found that increased paternal age is associated with a higher incidence of autism which provides evidence for new mutations as causative of ASD/PDD. A high predominance of males to females affected with autism has been well documented suggesting that mutations on the X chromosome may account for a number of affected individuals. Recent studies suggest that mutations on the maternal X chromosome may be causative based on data showing increased rates of psychiatric problems among mothers of children with autism, but not among fathers. Many mutations on the X chromosome arise during spermatogenesis due to the increased rates of DNA replication that occur in this process. The concept of new mutations occurring on the X chromosome during spermatogenesis in the maternal grandfather is known as the grandfather effect and accounts for several conditions such as Duchenne Muscular Dystrophy. We propose that increased maternal-grandpaternal age may be associated with an increased risk for autism in subsequent generations. We conducted anonymous surveys of families with children affected with autism to ascertain the ages of both sets of grandparents at the birth of each parent as well as geographical, employment, and military history. The paternal grandparents served as an internal control for the maternal grandparents. Of the 67 families successfully surveyed, we found that the average maternal grandfathers age was 2.5 years older than the paternal grandfather at the time of the parent's birth which was statistically significant. The data provides evidence for advanced maternal-grandpaternal age as a potential risk factor for the development of autism. Future studies should focus on the X chromosome and may also need to evaluate the environment of maternal grandparents for potential mutagens to explain the increased incidence of ASD/PDD seen in recent years.

An improved alpha-glucosidase enzyme for Pompe disease. *E. Kakkis[1], K. Antonsen[1], A. Cheng[1], T. Christianson[1], J. Femenia[1], B. Prince[1], M. Vellard[1], J. White[1], T. Zankel[2], V. Koppaka[1]* 1) BioMarin Pharmaceutical Inc. Novato, CA. 94949; 2) Raptor Pharmaceuticals Corp. Novato, CA. 94949.

Many Pompe patients with acid-alpha-glucosidase (GAA) deficiency have benefited from enzyme replacement therapy (ERT) using recombinant GAA (Myozyme, Genzyme Corp. Cambridge, MA) but a significant number do not achieve sufficient improvement. The efficacy of the GAA enzyme used may not be optimal due to low amounts of the high affinity N-linked oligosaccharide bis-mannose 6-phosphate mannose 7 (bisMan6P-Man7) needed for efficient uptake. Using a novel mutant CHO cell line, we have produced a highly phosphorylated GAA (BMN103) with fivefold higher bisMan6P-Man7 than Myozyme. The increased phosphorylation resulted in a 15-fold uptake efficiency improvement for BMN103 (Kuptake: 2 nM vs 30 nM for Myozyme) in Pompe fibroblasts that is comparable to other MPS enzymes used for ERT. Other GAA enzyme characteristics (Km, Vmax, Specific Activity, Sialic acid) were similar. When BMN103 was compared with Myozyme using 4 short IV infusions over 2 weeks in Pompe mice (3 month old; knockout, GAAtm1Rabn/J), BMN103 demonstrated 2-fold greater total enzyme activity compared to Myozyme in all tissues that were tested. BMN103 also reduced more of the accumulated glycogen in the target tissues both by total glycogen and by pathology. At a dose of 20 mg/kg, administration of BMN103 resulted in >85% reduction in glycogen in heart after only 2 weeks, while administration of Myozyme achieved only 50% reduction. At this dose, BMN103 achieved a ~50% glycogen reduction in diaphragm compared to only ~3% for Myozyme, a critical difference in a tissue required for respiratory function. In type-II skeletal muscle the 20 mg/kg dose of BMN103 showed ~ 25% clearance of glycogen while the same dose of Myozyme showed only <1% clearance. In a type-I skeletal muscle, 20 mg/kg BMN103 showed 30% glycogen clearance and the same dose of Myozyme only 13%. The enhanced phosphorylation and M6PR-mediated uptake, along with the dramatically improved clearance of glycogen from affected tissues, suggests that BMN103 may be a more effective GAA than Myozyme for treatment of Pompe Disease.

Genome-wide assessment of human-specific LINE-1 insertions. *A. D. Ewing[1], L. Zhang[1], V. G. Cheung[3], P. D. Sniegowski[2], H. H. Kazazian, Jr.[1]* 1) Genetics, University of Pennsylvania, Philadelphia, PA; 2) Biology, University of Pennsylvania, Philadelphia, PA; 3) Howard Hughes Medical Institute, Philadelphia, PA.

Human-specific LINE-1 elements (L1Hs) are the only active family of LINE-1 retrotransposons in the human genome. Throughout evolutionary history there has been a succession of L1 families which through their copy-and-paste replication mechanism are directly responsible for ~17% of genomic sequence. The active L1Hs family is still inserting new copies into the genome as evidenced by ~250 polymorphic insertion sites catalogued in several studies and at least 16 known cases of a de novo L1 insertion contributing to human diseases. These known polymorphic and private insertions are likely to represent only a small fraction of the total amount of genomic variation induced by L1 activity, much of which may be somatic. Results from mouse and rat models in our lab suggest that the majority of L1 retrotransposition events occur somatically and are therefore not transmissible to offspring. In order to confirm this phenomenon in humans, we analyze the genomic L1 content of monozygotic twins for differences. The presence of L1 insertions in one twin absent from the corresponding genomic location in the other twin is evidence of a somatic L1 retrotransposition event in the twin harboring the unique insertion. To address this possibility, we have developed an assay whereby the Human-specific LINE-1 content in the genome of a given individual can be assessed in a high-throughput manner, on a genome-wide scale. Following amplification of the 3 flanking sequences via a PCR-based approach, amplicon ends are sequenced on the Illumina Genome Analyzer platform yielding millions of reads per individual. Analysis of the sequencing results via our bioinformatics pipeline yields a number of interesting candidate somatic insertions for further validation. Furthermore, our results indicate that any given individual shares approximately 800 L1 insertion sites with the reference human genome and differs from this reference L1 content at ~200 loci. This technique allows us to study human L1 insertion sites on a genome-wide scale, enabling further studies of the impact of L1 activity on human disease.

The inadequacy of the Bonferroni correction in genome-wide association studies. *J. C. Wakefield* Statistics and Biostatistics, University of Washington, Seattle, WA.

The Bonferroni threshold is ubiquitous in genome-wide association studies, with the only source of contention being the number of tests that one must control for, accounting for the correlation between tests. A common target level is 5×10^{-8} (McCarthy et al, Nature Reviews Genetics, 9, 356-369), corresponding to 1 million tests and an level of 0.05. Using a Bayesian approach we show the prior over the collection of null hypotheses that corresponds to the Bonferroni correction, and argue that this prior is rarely realistic in a genome-wide setting. Even if one accepts that the control of the family-wise error rate is a reasonable criteria to adopt, a major problem with its use is the specification of a threshold for significance. In contrast to current practice, we show that the threshold should decrease with sample size, since a rule that is independent of sample size corresponds to a belief that the cost of type II errors decrease in importance as sample size increases, and in a manner which is implicit in the procedure, rather than being user-specified. We illustrate how power may be increased using a Bayesian approach and explicitly specifying both a prior on the effect size, and the costs of type I and type II errors. This approach revolves around the Bayes factor, which differs from the p-value by accounting for power. Hence a Bayesian threshold depends on sample size as well as the minor allele frequency. All derivations are based on an easily computable Bayes factor that requires only a confidence interval on the effect parameter for implementation (Wakefield, AJHG, 81, 208-227).

Effects of Genetic Background On ENS Development in the *Sox10^{Dom}* Model of Hirschsprung Disease. *L. C. Walters, V. A. Cantrell, E. M. Southard-Smith* Medicine and Cell & Developmental Biology, Vanderbilt University, Nashville, TN.

Abnormalities in development of enteric neural progenitors (ENPs) can lead to aganglionosis in a variable portion of the distal intestine, causing Hirschsprung disease (HSCR). Cumulative evidence suggests that variation in HSCR is due to gene interactions that modulate the ability of ENPs to populate the developing gut. *Sox10* is an essential gene for enteric ganglia development. *Sox10^{Dom}* mice on a mixed genetic background exhibit the variable aganglionosis seen in HSCR cases. We have established congenic lines of *Sox10^{Dom}* mice on distinct inbred genetic backgrounds, C57BL/6J (B6) and C3HeB/FeJ (C3Fe). These lines differ in penetrance and extent of aganglionosis. To define the impact of genetic background on processes during enteric nervous system ontogeny, we assayed these congenic lines for differences in migration, lineage potential, and proliferation of ENPs. Migration was evaluated by whole-mount immunohistochemistry. Both strains of *Sox10^{Dom}* mice displayed deficits in migration and decreased density of ENPs; however, the phenotype of B6.*Sox10^{Dom}* embryos was more pronounced. Differences in developmental potential were assayed by isolating enteric neural crest-derived stem cells (eNCSC) from *Sox10^{Dom}* congenic lines by flow cytometry for cell surface markers. eNCSC were cultured at clonal density, and immunohistochemistry was used to identify lineages within the resulting colonies. The number and type of colonies were quantified across multiple independent trials. We observed significant differences in lineage potential between the strains and genotypic classes. Proliferation was evaluated through immunohistochemistry of dissociated embryonic guts in culture. The significant differences between the *Sox10^{Dom}* congenic lines indicates that the deficiencies of ENP development leading to aganglionosis are not strictly due to migration defects. Our study of congenic lines contributes to understanding the effects of genetic background and mechanisms that impact variation in HSCR disease phenotype.

Prediction of regulatory elements in human histone deacetylases. *S. Khuri*[1,2], *Z. Jiang*[1] 1) Center for Computational Science, University of Miami, FL; 2) Dr John T Macdonald Fdtn Dept Human Genetics, Univ Miami Miller Sch Med, Miami, FL.

Human histone deacetylases (HDACs) remove acetyl groups from histone tails, thus repressing transcription. The 11 known human HDACs have an evolutionarily conserved catalytic domain, but differ in size, domain organization, and function. HDACs have some tissue and time specificities that have not been fully characterized. Recent evidence has shown that some HDACs may bind proteins other than histones. We used bioinformatics to gain a better understanding of how the HDACs themselves are regulated, and therefore of how they regulate gene expression in the human genome. We took the promoter region as the 2000bp flanking the transcriptional start site, and used several predictive methods to find possible active regulatory elements in these genes. We found that in HDACs 1, 3, 4, 5, 8, and 11, the highest density of predicted motifs was in the 500bp upstream of the TSS, whereas they were more widely spaced in other HDACs. We compared these against the promoter regions of HDAC homologs from different species using Footprint. The results show that the regulatory motifs are well conserved, and they clustered in a similar pattern on the promoter sequences of several mammals, indicating that the regulation of these genes is conserved across mammals. The motifs we found point to transcription factors that have some tissue and time preferences, which implies distinct expression patterns among the HDACs. In addition, we measured the accessibility of this 2000bp region using NXSensor (which predicts nucleosome exclusion sequences and thus accessibility of the transcriptional machinery to DNA). The accessibility scores correlated with the clustering pattern of the predicted motifs in several HDACs, further verifying that these motifs may well be active. In conclusion, we used motif prediction, footprint verification, and DNA accessibility mapping to identify putative active regulatory motifs in the promoter regions of human HDACs. These results can form the basis for laboratory experiments, and this integrated approach could be used for other protein families and metabolic pathways.

Genetic differentiation of genes involved in craniofacial anomalies between human populations. *R. Kimura, M. Osawa* Forensic Medicine, Tokai University School of Medicine, Kanagawa, Japan.

Modern humans show considerable intra- and inter-population variation in facial morphology. Although this common phenotypic variation is attributed to genetic factors, they have hardly been elucidated. In contrast, genes involved in craniofacial malformation have been clarified due to the recent advances in developmental biology and human genetics. Such genes might also be associated with the common variation. Furthermore, the inter-population variation can play a key role in effectively picking out the most likely candidate genes. If we hypothesize that phenotypic differentiation between populations has been accelerated by positive selection, causal genes are expected to show genetic differentiation. In this study, considering craniofacial anomalies (CFA)-related genes (201 genes) as candidates, we examined genetic differentiation between African (YRI), European American (CEU), and East Asian (CHB+JPT: EAS) populations using the HapMap Phase II data. As an indicator of genetic differentiation, maxFST, that is the maximum of single-locus FST values in a 100 kbp window, was used. As results, high differentiation was observed in windows including ADAMTS2, PAFAH1B1, SNRPN (YRI vs CEU and YRI vs EAS), GLI3 (YRI vs CEU and CEU vs EAS), EDAR (YRI vs EAS and CEU vs EAS), AGPS, PTHR1, COL5A1, GH1, TWIST1, ENAM (YRI vs CEU), MAP2K2, GJB6, COL11A1, ERCC2, and FOXL2 (YRI vs EAS), FBN1, FLNB, PTPN11, INSR, and LMNA (CEU vs EAS). Between YRI and CEU, maxFST values in windows including CFA-related genes were elevated compared with those in windows including other genes, implying that some CFA-related genes have been subjected to recent positive selection. Of course, future association studies are indispensable to identify genes associated with the common variation in facial morphology. Taken into account the statistical limitation of large scale association studies, observations in this study would greatly help us to accomplish our goal efficiently.

Genetic linkage of measures of hand joint structure and osteoarthritis to chromosomes 7p and 14q. *D. L. Duren[1], R. J. Sherwood[1], J. Blangero[2], J. Subedi[3], T. Dyer[2], B. Jha[4], J. L. VandeBerg[2], B. Towne[1], S. Williams-Blangero[2]* 1) Lifespan Hlth Res Ctr, Wright State Univ Boonshoft Sch Med, Dayton, OH; 2) Southwest Foundation for Biomedical Research, San Antonio, TX; 3) Miami University, Oxford, OH; 4) Institute of Medicine, Tribhuvan University, Kathmandu, Nepal.

Osteoarthritis (OA) is a multi-factorial disease with a strong genetic component. The specific genetic underpinnings of OA, however, have yet to be identified. Because the OA phenotype is comprised of a set of multiple distinct sub-phenotypes (e.g., joint space narrowing and osteophyte formation), each may be under unique genetic influence. We present evidence for significant genetic effects on joint space and overall grades of OA in the hand joints of 304 subjects from a single, large extended family in Nepal. All 304 of these individuals have been genotyped for some 400 autosomal markers spaced approximately every 10 cM. A variance components-based linkage analysis method (SOLAR; Almasy and Blangero, 1998) was used to obtain multipoint LOD scores. Linkage analysis of these measures of joint health and structure revealed two significant LOD scores (> 3.0) for linkage to markers on chromosomes 7 and 14. One significant linkage for hand joint space grade (LOD = 3.08) was found on chromosome 7p between markers D7S507 and D7S493, which overlap regions 7p21.1 and 7p15.3. This region is very near those identified by other groups to be associated with hand OA phenotypes. The second linkage (LOD = 3.00) was for a global score of hand OA, and was found on chromosome 14q near marker D14S292, which corresponds to region 14q32.33. Candidate genes of interest in the significant linkage regions include SP8, Twist1, and AKT1. Twist1 is of notable interest in that it inhibits chondrocyte gene expression through the Wnt signaling pathway and is differentially expressed in bone tissue of patients with OA. The identification of linkage to these two regions harboring genes involved in cartilage and/or bone is both promising and exciting. Future work will seek to identify other specific genes that influence the health of the joint tissues of hands. Supported by NIH grants R01 AI37901, R01 AI44406, R01 HD40377.

Management trials of exfoliative cutaneous lupus erythematosus in German shorthaired pointers with cyclosporine, hydroxychloroquine and adalimumab. *E. A. Mauldin[1], D. O. Morris[2], M. L. Casal[2]* 1) Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA; 2) Clinical Studies, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA.

Cutaneous lupus erythematosus (CLE) is well-characterized in humans and is divided into three major categories: chronic CLE (includes localized and generalized discoid lupus), subacute LE and acute LE. We have developed a colony of German shorthaired pointer (GSP) dogs with exfoliative cutaneous lupus erythematosus (ECLE), which appears to be quite similar clinically and histologically to chronic CLE in humans. Pedigree analysis of dogs with ECLE is highly suggestive of an autosomal recessive trait. Six affected dogs were enrolled in the study (2 females and 4 males) ranging from 6 weeks to 2.3 years of age at study begin. Five affected GSPs received immunomodulatory drugs sequentially to alleviate clinical signs (lameness, erythema, scaling, erosions/ulcers). Five dogs received oral cyclosporine (2.5-10 mg/kg/q24h) for a minimum of 4.5 months and maximum of two years. In four dogs, the drug was combined with ketoconazole (5-7 mg/kg/q24h). Cyclosporine decreased joint pain and stiffness and provided temporary improvement of some skin lesions but did not halt disease progression. Three dogs received hydroxychloroquine 5 to 10mg/kg/q24h for six weeks, 7 months, and 9 months, respectively, with no side effects (weekly CBC/serum biochemistry screens, normal EKG). Hydroxychloroquine mildly ameliorated clinical signs in the two dogs on extended treatment and normalized globulin levels in all three dogs. Four dogs were euthanized between one and three years of age due to disease progression. The two remaining male dogs (donated at > 1yr age) received a commercial human monoclonal TNF- antagonist, adalimumab at 0.5 mg/kg q2wks sc for 12 weeks. Biweekly CBC/serum biochemistry screens were within normal limits. A temporary improvement was seen in both skin lesions and lameness. None of the drug therapies provided long-lasting improvement. However, dogs with this heritable form of what appears o be chronic CLE in humans provide a unique model to study disease progression and treatment trials.

The Frequency of a Polymorphism in the Glutamate Cysteine Ligase Gene Correlates With Malarial Prevalence in Populations of African Descent. *T. Le[1], G. Ayodo[2], J. Canter[3], D. Reich[2], M. Summar[3]* 1) Pediatric Critical Care Medicine, Vanderbilt Children's Hospital, Nashville, TN; 2) Department of Genetics, Harvard Medical School, Boston, MA; 3) Center for Human Genetic Research, Vanderbilt University Medical Center, Nashville, TN.

Background: Glutamate cysteine ligase (GCL) is the rate-limiting enzyme in the glutathione biosynthesis pathway, and genetic variation in this gene can affect the ability to produce glutathione and respond to oxidative injury. We previously identified a non-synonymous polymorphism (C1384T) present only in individuals of African descent and demonstrated that this polymorphism encodes an enzyme with no activity in vitro. Objective: To determine whether the 1384T polymorphism is correlated with development of severe malaria in populations of African descent. Methods: Using high-throughput genotyping, we have genotyped Kenyans from malaria-endemic and non-endemic regions as well as several African-descent populations. Results: In a malaria-endemic region of Kenya, the 1384T polymorphism is found in 7.8% of individuals. In contrast, within a non-endemic region of Kenya, the 1384T polymorphism is present in only 3.8% of the population ($p<0.05$). Similarly, individuals from a non-endemic region of Ghana have a 5% prevalence of this allele, whereas African-Americans from North Carolina exhibit 7.4% prevalence. Within the endemic regions we studied, we note that the 1384T allele is present in 6.3% of patients with severe malaria, whereas it is present in 9.0% of controls. Though not statistically significant, these results suggest the 1384T polymorphism is associated with less severe malarial disease. Conclusions: We have previously described a glutathione production pathway polymorphism common in individuals of African descent with little or no in vitro activity. Here, we demonstrate an increased frequency of the 1384T GCLC allele among malaria endemic populations as compared to populations from non-endemic areas. In addition, our results suggest that this polymorphism is associated with less severe disease.

Oliogenic analysis locates several triglyceride metabolism genes in the GOLDN study. *E. W. Daw[1], I. B. Borecki[1], A. VanBrunt[1], J. M. Ordovas[2], M. Y. Tsai[3], P. N. Hopkins[4], J. Hixson[5], M. A. Province[1], D. K. Arnett[6]* 1) Washington Univ, St Louis, MO; 2) Tufts Univ, Boston, MA; 3) Univ of Minnesota, Minneapolis, MN; 4) Univ of Utah, Salt Lake City, UT; 5) UT Health Science Center, Houston, TX; 6) Univ of Alabama, Birmingham, AL.

The GOLDN study examined the Genetics of lipid metabolism and response to treatment in 201 families, ranging in size from 53 to 3. 1027 subjects completed the Post-prandial Lipoprotein arm of the study: after fasting, subjects were asked to drink a fatty shake and Lipid levels were measured just prior to drinking the shake, at 3.5 hours, and at 6 hours after drinking the shake. We have examined the Triglyceride (TG) levels at each time point, as well as the slopes between time points and the area under the curve to search for genes contributing to TG Metabolism. Both raw TG values and log transformed TG values were examined. Microsatellite markers were typed in over 2000 family members, including a number of markers typed in additional family members in the predecessor study. Using all these markers improved meiotic inference. We conducted an oligogenic simultaneous segregation and linkage analysis with Monte Carlo Markov chain (MCMC) methods for each TG measurement, and identified several linked regions. The strongest signals were obtained on chrs 4, 11, and 17, with LOP values > 4 (Daw et al., Genet Epidemiol, 24, p181-90,2003). The chromosome 4 and 11 genes are linked to several TG measures, while the chromosome 17 gene is linked primarily to TG uptake. In the chr 4 and 11 regions, SNPs were typed in previously identified candidate genes. However, these genes were on the edge of the linked region, and MCMC combined segregation and linkage analysis did not find any of these SNPs to be strongly associated with the linkage peak. Thus, we conclude that there are genes other than the previously identified candidate genes involved in lipid metabolism in these regions. In addition, these results demonstrate the utility of MCMC oligogenic simultaneous segregation and linkage analysis combined with association testing of candidate genes for pharmecogenetic traits.

A High-Density Screen of 25 Genes Identifies Adiponectin as a Candidate Locus for Skeletal Muscle Fat Accumulation. *I. Miljkovic[1], L. M. Yerges[1], C. L. Gordon[2], B. H. Goodpaster[1], C. M. Kammerer[1], L. H. Kuller[1], C. H. Bunker[1], A. L. Patrick[3], V. W. Wheeler[3], J. M. Zmuda[1]* 1) University of Pittsburgh, Pittsburgh, PA, USA; 2) McMaster University, Hamilton, Ontario, Canada; 3) Tobago Health Studies Office, Scarborough, Tobago, West Indies.

Adipose tissue in skeletal muscle has recently been identified as an important fat depot that increases with aging, contributes to the development of diabetes, and is greater in individuals of African origin compared with Caucasians. However, despite the fact that it is a heritable trait, specific genetic factors contributing to skeletal muscle fat remain to be defined. The aim of this study was to examine the potential genetic contributions to peripheral quantitative computed tomography (pQCT) measured intermuscular adipose tissue (IMAT, expressed in mm^2) in 471 individuals aged 18 to 103 years belonging to 8 large Afro-Caribbean families (median family size 51 individuals; 3535 relative pairs). We systematically screened 768 tag and potentially functional single nucleotide polymorphisms (SNPs) in 25 genes, which have previously been associated with lipoprotein metabolism or were identified as potential candidate genes in animal studies. After adjusting for age, gender, height, total adiposity as measured by whole body dual-energy X-ray absorptiometry (DXA) percent total body fat, and muscle area, 7 genes showed nominal associations with IMAT ($P<0.05$). The most significant association was with an intronic tag SNP rs3821799 (MAF=43%) in adiponectin (ACDC), a gene whose protein product promotes fatty acid oxidation, increases insulin sensitivity and promotes glucose uptake in skeletal muscle. Individuals with two copies of the minor allele for rs3821799 had ~25% higher IMAT (additive $P=0.008$) compared to those with two major alleles (adjusted means SE: 209.227.5 versus 262.436.2 mm^2). This SNP accounted for ~1% of the total phenotypic variability in IMAT. These findings require confirmation, but suggest a novel association between common variants in adiponectin gene and the distribution of ectopic fat in skeletal muscle.

MPS BRAZIL-NETWORK: 4 YEARS IMPROVING DIAGNOSIS AND MANAGEMENT OF MUCOPOLYSACCHARIDOSES IN BRAZIL. *I. Schwartz, R. Giugliani, A. Federhen, L. Pinto, S. Leistner-Segal, U. Matte, M. Burin, J. Coelho, E. Valadares, A. Duarte, C. Steiner, C. Kim, A. Martins, D. Horovitz, M. Ribeiro, R. Boy, A. Acosta, J. Pina Neto, E. Ribeiro, L. Silva* Medical Genetics Service, Hospital Clinicas de Porto Alegre, Porto Alegre RS, Brazil.

Aim: To present the epidemiological data generated by MPS-BRAZIL NETWORK, a collaborative partnership among centers from different Brazilian regions aiming to improve the diagnosis and management of MPS diseases. Methods: The Medical Genetics Service of Hospital de Clínicas de Porto Alegre is the coordinating center, providing the information on the management of patients and making available the laboratorial tests necessary for their diagnosis. Results: 1)Since the MPS-BRAZIL NETWORK started operations in 2004 the rate of diagnosis of MPS performed by the coordinating center increased from 1.97 patients/mo to 6.6 patients/mo; 2)MPSII appears to be the most frequent type of MPS in Brazil; 3)MPSI is more common in the South and Southeast regions,while MPS VI seems to be less frequent in the South region; 4)MPSIII seems to be underdiagnosed in Brazil; 5)The higher prevalence of MPSVI in Northeast is probably explained by the combination of founder effect and endogamy; 6)Mean age at diagnosis was found to be high in all the types of MPS,especially in Northeast; 7)The rate of recurrence of MPS in the same family was found to be higher in Northeast. Conclusions: The MPS BRAZIL NETWORK is improving the diagnosis of MPS in Brazil.Economic and cultural aspects are still important contributors to the epidemiology,since it was found differences among Brazilian regions in relation to age at diagnosis,rate of consanguinity and rate of recurrence of MPS. Support: CNPq, BioMarin, Shire, Genzyme, LOREAL, Brazilian Academy of Sciences, UNESCO.

A targeted association study in Systemic Lupus Erythematosus (SLE) identifies novel risk alleles, and replicates known susceptibility alleles. *J. Lian[1], P. Goyette[1], R. Graham[2], J. Wither[3], P. R. Fortin[3], J. D. Rioux[1,2,4], GenES/CaNIOS Investigators* 1) Research Center, Montreal Heart Institute, Montreal, Quebec, Canada; 2) Program in Medical and Population Genetics, Broad Institute of Harvard and the Massachusetts Institute of Technology, Cambridge, Massachusetts, USA; 3) Division of Rheumatology and Toronto Western Research Institute, University Health Network; University of Toronto, Toronto, Ontario, Canada; 4) Department of Medicine, University of Montreal, Montreal, Quebec, Canada.

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease associated with the abnormal production of autoantibodies directed mostly against nuclear antigens. Although the etiology of the disease is not fully known, it is believed that genetic, hormonal and environmental factors contribute to susceptibility. Epidemiological studies have revealed a significant genetic impact on the pathogenesis of SLE. Recent candidate-gene and genome-wide association studies (GWAS) have identified several susceptibility genes for SLE. The current study aims to replicate these previously reported risk alleles for SLE, as well as evaluate risk alleles recently identified in other complex immune disorders, including IRGM, IL23R, ATG16L1, IBD5, CARD15 and PTGER4 in Crohns disease (CD), TNFAIP3 in rheumatoid arthritis (RA), and IL2R, IL7R, PTPN2, CTLA4 and ERBB3 in Type 1 Diabetes (T1D). The study was performed using a cohort of 270 well-phenotyped Canadian SLE trios, mostly of European descent, collected through the GenES/CaNIOS Consortium. Our results replicate several previously reported associations and identify novel associations to SNPs in genes previously reported in RA and T1D. A stratification of the data for renal involvement suggests that some of these markers may be associated with specific SLE subphenotypes. The current study confirms the existence of multiple risk alleles for SLE, and suggests that some risk factors for SLE may be shared with other autoimmune disorders.

Evaluation of mechanically enhanced alginate-based capsule for cellular gene therapy. *M. Mazumder[1], F. Shen[2], N. Burke[1], H. Stöver[1], M. Potter[2]* 1) Chemistry, McMaster University, Hamilton, Ontario, Canada; 2) Pathology & Molecular Medicine, McMaster University, Hamilton, Ontario, Canada.

Microencapsulated cells engineered to secrete therapeutic proteins have been effectively applied in treating mouse models of several genetic disorders such as dwarfism, lysosomal storage diseases, and hemophilia. The most commonly used and studied capsules are alginate-Poly-L-lysine-alginate capsules (designated APA capsules), which have suitable biocompatibility and permeability for implantation. A concern with APA capsules is the loss of structural integrity during implantation due to loss of ionic ally bonded calcium, essential for stability of the alginate core. Our objective was to find a biocompatible covalently linked alternative to the APA capsule. In our study, poly(sodium methacrylate-co-2-(methacryloyloxy)ethyl acetoacetate) (designated A70), was introduced to the alginate core of the capsules (designated composite capsules). The A70 reacts with poly-L-lysine (PLL) to form a covalent interpenetrating polymer network, with the distribution of the covalent network depending on the A70/PLL ratio and the molecular weight of the polymers as demonstrated by confocal microscopy using FITC labelled A70 or PLL. In vitro tests of mechanical stability showed that 100% of the composite capsules were intact after shaking in dd-H2O for 3 hours, and ~40% of the capsules were intact after shaking in 0.003% EDTA for 15 minutes, while regular APA capsules totally collapse with both kinds of treatment. The cell viability of the capsules, as demonstrated by alamar blue staining, is decreased by 20% when A70 is included in the core. However, post-encapsulation the average cell number per composite capsule increased at the same rate as the cells in APA capsules. Permeability of the composite capsules was assessed by incubating the capsules with a series of different molecular weights of FITC-labelled dextrin under confocal microscopy. The cut-off size of the composite capsule was approximately 150kDa, similar to APA capsules. This preliminary data indicates that composite capsules are significantly stronger than APA capsules while maintaining suitable biocompatibility and permeability.

Candidate gene identification and analysis in Toriello-Carey Syndrome. *J. C. Yedowitz[1], D. H. Tegay[1, 2], H. V. Toriello[3], E. Hatchwell[2]* 1) Internal Medicine, New York College of Osteopathic Medicine at NYIT, Old Westbury, NY; 2) Stony Brook University Medical Center, Stony Brook, NY; 3) Spectrum Health, Grand Rapids, MI.

Toriello-Carey Syndrome (TCS; OMIM#217980) is a rare multiple congenital anomaly syndrome characterized by corpus callosum agenesis, cardiac defects, Robin sequence, developmental delay, hypotonia and dysmorphic facial features (including micrognathia, ear anomalies, telecanthus or hypertelorism, small nose and full cheeks). Although the phenotype is well established, the cause of TCS remains unknown.

Previously, using aCGH on 24 subjects diagnosed with Toriello-Carey syndrome, we reported the detection of 4 pathologic submicroscopic de-novo CNVs (one ~5Mb deletion at 6q14.2-15, one ~7Mb deletion at 1q41-42.3 and two ~5Mb deletions involving an overlapping region at 22q12), implicating 3 distinct loci in TCS pathogenesis. In addition a fourth locus was reported in a TCS subject with a balanced de novo translocation directly interrupting the SATB2 gene. We have focused our recent work on identifying candidate genes for TCS from within these regions using hierarchical clustering based on expression pattern similarity to SATB2.

For the identified candidate genes, including RFPL-1 (Ret-finger protein-like 1; OMIM#605968) at chromosome 22q12, having the greatest degree of expression homology to SATB2, we will present sequencing and deletion/duplication data obtained from our cohort of 24 TCS subjects.

Fine-Mapping of the Chromosome 10q.21 Linkage Region in Alzheimers Disease Cases and Controls. *D. Avramopoulos[1,2], K. H. Miller[3], M. Szymanski[2], R. Wang[2], S. S. Bassett[1], M. D. Fallin[3]* 1) Dept Psychiatry, Johns Hopkins Univ, Baltimore, MD; 2) McKusick Nathans Inst. of Genetic Medicine, Johns Hopkins Univ, Baltimore, MD; 3) Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD.

Many studies including our own have reported genome wide significant linkage for Alzheimers disease on chromosome 10. Our linkage results showed a strong parent of origin effect that was consistent in two independent data sets, where linkage was present in families with an affected mother. We have now fine mapped the region of interest on 349 cases (104 with an affected mother) and 289 controls by genotyping ~4,500 Single Nucleotide Polymorphisms (SNPs) across a 17.3 Mb interval. The SNPs were selected to provide a complete representation of all variation genotyped by the HapMap project with a pair wise SNP correlation of r-squared = 0.8 or better. We found no single common variant of an effect size sufficient to produce the linkage result. We identify 3 regions that show a high concentration of positive signals, two of which also show strong parent of origin effects consistent with the linkage results. One is a gene devoid region adjacent to the GDF10 gene and to an array of low copy repeats containing multiple genes, the second region includes the PRKG1 gene while the third includes the PCDH15 gene. Additionally a SNP close to the Acetylcholine transporter gene showed a strong signal in male carriers of the APOE e4 allele (p=10-5), an interesting result given the prior evidence for this gene. In conclusion, our dense fine mapping, in agreement with the results of recent less dense genome wide association studies, shows no single common variant responsible for the chromosome 10 linkage signal. Rather, our data point to a number of interesting sub-regions that might harbor multiple risk alleles contributing to the disease.

EphB2 sequence variation and prostate cancer risk in African American men. *W. Hernandez[1], C. Robbins[2], J. Carpten[2], R. A. Kittles[1]* 1) Dept of Medicine, University of Chicago, Chicago, IL; 2) Division of Integrated Cancer Genomics, Translational Genomics Research Institute, Phoenix, AZ.

The Eph receptors and the ephrin family of ligands are involved in many developmental functions including angiogenesis. The receptors are signaling molecules composed of an extracellular domain-motif typically found in adhesion molecules and a conserved tyrosine kinase domain in the cytoplasmic region which is flanked by a less conserved juxtamembrane region. Several variant forms of Eph receptors have been identified, including EphB2, which originate from alternative splicing and may have different functional properties. Deregulation of EphB2 has been implicated in the development and progression of several types of cancer, including prostate cancer (PCA). Here we performed re-sequencing of EphB2 and then a case-control association study on EphB2 polymorphisms to investigate their role in prostate cancer among African Americans. SNP discovery occurred in a set of 48 AA sporadic PCA cases. All coding exons of EphB2 were screened for sequence variants by DNA re-sequencing. In addition we supplemented discovered variants with random evenly spaced SNPs selected from the International HapMap Project to encompass the entire EphB2 transcription unit including introns and 5kb of upstream and downstream untranslated sequences. We genotyped 320 markers in 490 prostate cancer cases and 572 controls and identified common EphB2 SNPs significantly associated with prostate cancer. Empirical P-values that corrected for multiple testing were generated using the adaptive permutation approach. For several SNPs, rs4612601, rs12117711, rs2817897, and rs4600017, homozygotes for each minor allele had at least a two-fold increased risk of PCA ($P < 0.00001$). Haplotype analyses confirmed the single marker associations. Our data further demonstrates that variants of EphB2 are associated with prostate cancer risk in African American men.

Large-Scale Transcriptional Profiling and Genome-Wide Association of Intelligence in the Genetics of Brain Structure and Function Study. *J. Charlesworth[1], J. E. Curran[1], M. Carless[1], M. P. Johnson[1], H. H. H. Göring[1], T. D. Dyer[1], L. Almasy[1], P. T. Fox[2], R. Duggirala[1], E. K. Moses[1], D. C. Glahn[2], J. Blangero[1]* 1) SFBR, San Antonio, TX; 2) UT Health Science Center, San Antonio, TX.

The genetic architecture of general intelligence is complex, involving interactions between multiple genetic and environmental factors; however the specific genes involved are largely unknown. In this study, we employ an integrative genomic approach that utilizes large-scale transcriptional profiling and genome-wide association to identify novel genes influencing general intelligence. This study is being conducted on 1,000 individuals from 40 Mexican American families previously investigated as part of the San Antonio Family Heart Study. As collection is ongoing, the data provided here are from the first 453 individuals. Intelligence as measured by the WASI 2-subset IQ test showed a significant heritability of 0.865 ($p=2.8x10^{-20}$). We genotyped individuals using the Illumina HumanHap 550K beadchip and obtained genome-wide transcriptional profiles (Illumina Human 6v1) using RNA extracted from lymphocytes obtained approximately 15 years prior to IQ testing. We identified 633 transcripts whose expression was significantly correlated with IQ at a false discovery rate of 10%. Zinc ion binding is significantly enriched in this set (80 genes; $p=8.3\times10^{-7}$), with the three most significantly correlated genes (*ZNF618*, *ZBED3* and *WDFY3*) all related to zinc binding and a total of 31 zinc-finger proteins in the set. Given the prospective nature of the study design, these results suggest a striking role of transcriptional variation in cognitive function. Early results from the genome-wide association analysis of IQ reveal three suggestive regions of association, with multiple associated SNPs in each region. The first is at 18q22.3 near *CBLN2*, the second at 3p26.3 near *LRRN1* and the third at 6p24.1 near *HIVEP1* and *EDN1* (a region previously identified as harboring both an IQ-related QTL and a schizophrenia susceptibility locus). We hope that unraveling the genetic architecture of intelligence will offer insight into related conditions such as cognitive dysfunction, learning disabilities and degenerative disorders.

Intragenic deletion in FLNA causes periventricular nodular heterotopia. *I. Coupry[1], D. Lacombe[1,2], G. Sole[1], E. Guérineau[1], C. Rooryck-Thambo[1,2], S. Dubois[1], F. Martins[1], N. Phillip[3], L. Villard[4], B. Arveiler[1,2], C. Goizet[1,2]* 1) Lab of Hum Genet, Univ Victor Segalen, Bordeaux 2, Bordeaux, France; 2) Dep of Med Genet, Hôpital Pellegrin, Bordeaux; 3) Dep of Med Genet, Hôpital Timone-Enfants, Marseille; 4) INSERM U491, Marseille, France.

Filamin A, encoded by the FLNA gene located on chromosome Xq28, is a cytoskeletal protein that cross-links actin in a regulated fashion. Mutations in FLNA have been associated with bilateral periventricular nodular heterotopia (BPNH) and also with other various phenotypes including oto-palato-digital syndrome type 1 and 2 (OPD), Melnick-Needles syndrome (MNS) and frontometaphyseal dysplasia (FMD). We have analyzed the FLNA gene in 50 patients with characteristic signs of BPNH on brain MRI. Using DHPLC and sequencing, we identified a total of 18 different point mutations predominantly leading to a truncating protein. All mutations except one were novel. Alternatively, we developed gene dosage analysis using Quantitative Multiplex Fluorescent PCR (QMF-PCR). QMF-PCR was used for analyzing 10 patients negative for a FLNA point mutation. We identified an intragenic deletion suppressing exons 31-48 in one patient displaying BPNH associated with Ehlers-Danlos syndrome. Although the frequency of such gene dosage anomalies needs to be evaluated in a larger population, our preliminary data reveal that gene dosage analysis of FLNA should be considered in the molecular diagnosis strategy of BPNH.

Comparison between targeted and targeted plus whole genome aCGH platforms - A pilot study of 35 cases. *B. A. Brown-Kipphut, L. Li, N. Wang, M. A. Iqbal* Cytogenetics/Pathology, University of Rochester, Rochester, NY.

Objective: Compare and contrast the targeted array against the targeted plus whole genome array using BAC and Oligo aCGH platforms. Methods: 35 cases (12 normal) and (23 abnormal) were included in the study. Nine normal cases were determined by chromosome analysis in our laboratory and 3 had previous normal aCGH results at other institutions. Abnormal cases included: 1) 5 cases determined by chromosome analysis in our laboratory, 2) 11 cases using DNA from the Coriell Institute, and 3) 4 cases with previous abnormal aCGH results at other institutions. To examine clinical sensitivity and specificity of different BAC and Oligo aCGH chips, genomic DNA was processed and analyzed according to manufacturers guidelines using a) Perkin Elmer targeted constitutional chip (CC) v3.0 (30 cases) b) Perkin Elmer targeted plus whole genome v4.0 (5 cases) and c) custom Agilent 4x44 targeted plus whole genome oligo aCGH chip (4 cases). Fluorescent in situ hybridization (FISH) was used to confirm abnormal aCGH findings. Results: Twenty-nine out of 30 samples (15 normal and 15 abnormal) analyzed by targeted CC v3.0 confirmed previous results; however, one developmental delay case with a 2q23-2q31 duplication was missed. Four out of 5 abnormal samples including dup 2q analyzed by the targeted plus whole genome CC v4.0 were confirmed; however, one abnormal case with a marker chromosome was missed. Four samples previously identified as normal by CC v3.0 and a BAC emulated oligo aCGH chip (custom Agilent 4x44) from another institution were evaluated on a custom Agilent 4x44 targeted plus whole genome oligo aCGH chip. The analysis confirmed normal findings in 3 out of 4 cases. In one case with autism previously reported as normal by the BAC emulated oligo aCGH platform, a ~0.5Mb duplication, which included three genes (PRMD1, MLT4, and DAC2) at chromosome 6q27 subtelomere region, was identified. Conclusion: 1) Targeted plus whole genome oligo aCGH platforms are more sensitive in detecting copy number abnormalities. 2) aCGH analysis should always be used after routine chromosome analysis is performed.

Genome-wide Association Mapping in Multiplex Autism Families. *L. A. Weiss[1], D. E. Arking[2], T. Green[1], J. F. Gusella[1], S. L. Santangelo[1], R. E. Tanzi[1], P. Sklar[1], A. Chakravarti[2], M. J. Daly[1], Autism Consortium Gene-Finding Group & Autism Genome Project* 1) Ctr Human Genetic Research, Harvard-MGH/Broad, Boston, MA; 2) Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD.

Autism is a highly heritable developmental disorder, though to date, identifying specific genes has met with limited success. We therefore initiated the first genome-wide association mapping study using 500,000 SNP markers in multiplex autism families. (Linkage analysis is reported in an abstract by Arking et al.) Our primary datasets were ~800 AGRE families genotyped on Affymetrix 5.0 at the Broad Institute, and an NIMH autism sample (250 families independent from AGRE), genotyped on the Affymetrix 500K/5.0 at Johns Hopkins University. Combining these datasets using stringent QC measures yielded 864 complete families with 1,594 trios. Data analysis was performed using PLINK. Initial TDT analysis did not yield genome-wide significant association. However, we utilized additional SNP microarray follow-up data, as well as designing Sequenom assays in our top hits for genotyping by the Autism Genome Project. In the combined dataset, an array SNP on chromosome 5p15 shows genome-wide significant association with autism. We followed up these results by performing imputation analysis to capture additional variation, identifying signals of interest that were validated by Sequenom genotyping. We also looked at positional and functional candidate regions previously implicated for autism, such as genes for Mendelian disorders associated with autism, genes containing rare variants associated with autism, regions of copy number variants associated with autism, and candidate genes for association between common variation and autism. The only region meeting criteria for region-wide association is the Williams Syndrome region on chromosome 7. Our results suggest that individual common variants of major effect do not explain a large proportion of the heritability of autism, nor do previously studied candidate genes. We report here a novel association of genetic variation on 5p15 with autism. This signal falls between *SEMA5A* and *TAS2R1*.

Genetic History of human populations of East African inferred from mtDNA and Y chromosome analyses. *J. Hirbo[1], S. Omar[2], M. Ibrahim[3], S. Tishkoff[4]* 1) Dept. of Biology, University of Maryland, College Park, MD; 2) KEMRI, Centre for Biotechnology Research and Development, Nairobi, Kenya; 3) Dept. of Molecular Biology, Institute of Endemic Diseases, University of Khartoum, Khartoum, Sudan; 4) Department of Genetics and Biology, University of Pennsylvania, Philadelphia.

Evidence from genetic, paleobiological, and archaeological studies suggest that Africa, especially East Africa, is most likely to be the cradle of the modern human species. Despite this fact, very little is currently known about genetic diversity in African populations in general, and East African populations in particular. Genetic data demonstrate that the patterns of genetic variation in East African populations are complex. All four major language families spoken in Africa (Afro-Asiatic, Nilo-Saharan, Niger-Kordofanian, and Khoisan) are found in the region. As part of a large study of population genetic diversity of East and Northeast Africa, we examined Y chromosome genetic diversity (to ascertain paternal lineages) as well as mitochondrial genetic diversity (to ascertain maternal lineages) in 1200 - 1500 individuals from ~ 40 Tanzanian, Sudanese, and Kenyan populations. For the Y chromosome analysis, we genotyped 60 UEPs (analyzed in a hierarchical manner to construct haplotypes) in a total of ~1500 male individuals. In order to infer ages of lineages and migration patterns, we further genotyped the individuals for 16 Y chromosome microsatellites. For the mtDNA analysis, we sequenced the mitochondrial D-loop in a total of 1200 individuals from the same populations, and for 200 individuals, we did complete mitochondrial genome sequencing. We compare our results with published results of studies from other parts of Africa and the Middle East. Our results indicate that East African populations have some of the most ancestral Y chromosome and mtDNA lineages in Africa, suggesting that they may have been an ancient source of dispersion throughout Africa. Additionally, we find evidence for ancient geneflow between East Africa and the Middle East. We also ascertained the effect of the Bantu-expansion and signature of recent migration of Cushitic-speaking groups originating from Ethiopia on peopling of East Africa.

Termination of damaged protein repair defines the occurrence of symptoms in carriers of the m.3243A>G tRNA-Leu mutation. *H. Smeets[1], R. van Eijsden[1], L. Eijssen[1], P. Lindsey[1], C. van den Burg[1], L. de Wit[2], M. Rubio-Gozalbo[3], C. de Die[1], T. Ayoubi[1], W. Sluiter[2], I. de Coo[2]* 1) Dept Gen & Cell Biol, Univ Maastricht, Maastricht, Netherlands; 2) Department of Biochemistry, Mitochondrial Research Unit, Erasmus MC, Rotterdam - The Netherlands; 3) Department of Paediatrics and Laboratory Genetic Metabolic Diseases, Maastricht University Hospital, Maastricht - The Netherlands.

The m.3243A>G mutation in the mtDNA tRNALeu gene displays a heterogeneous phenotypic expression. It is the most frequent cause (80%) of the MELAS syndrome (Mitochondrial Myopathy, Encephalopathy, Lactic Acidosis and Stroke-like episodes), but it can also lead to type 2 diabetes, deafness, renal tubulopathy and/or cardiomyopathy. To identify pathogenic processes induced by this mutation, we compared global gene expression levels of muscle biopsies from affected and unaffected mutation carriers with controls. Gene expression changes were relatively subtle. In the a symptomatic group 200 transcripts were up- and 12 were down-regulated, whereas in the symptomatic group this was 15 and 52 respectively. In the a symptomatic group, oxidative phosphorylation (OXPHOS) complex I and IV genes were induced. Protein turnover and apoptosis were elevated, most likely due to the formation of dysfunctional and reactive oxygen species (ROS) damaged proteins. These processes returned to normal in symptomatic patients. Components of the complement system were up-regulated in both groups, which might indicate muscle regeneration. Most likely, protein damage and OXPHOS dysfunction stimulate repair (protein regeneration) and metabolic adaptation (OXPHOS). In a symptomatic individuals these processes suffice to prevent the occurrence of symptoms. However, in affected individuals the repair process terminates, presumably because of excessive damage, and switches to muscle regeneration, as indicated by a stronger complement activation. This switch leaves increasingly damaged tissue in place and muscle pathology becomes manifest. Therefore, the expression of complement components might be a marker for the severity and progression of MELAS clinical course.

Identification of an X-Linked locus associated with acute rejection in kidney transplant patients. *W. S. Oetting[1], S. Basu[2], M. J. Brott[1], K. R. Linnihan[1], A. J. Matas[3]* 1) College of Pharmacy, Univ Minnesota, Minneapolis, MN; 2) Div. of Biostatistics; 3) Dept. of Surgery.

DNA variants associated with solid organ transplant outcome were identified using a custom chip containing 3,590 single nucleotide polymorphisms (SNPs), many of which are thought to be functional variants within biologically relevant genes including genes in pathways associated with immunity, cell signaling, ADME, cell growth and proliferation. Additional haptag and admixture SNPs were included. Blood was obtained with informed consent and DNA isolated from 271 kidney allograft recipients, 136 of whom had acute rejection (AR) within 6 months of transplant, and 135 of whom did not have any detectable AR after at least 8 years post-transplant. All received Ab induction and CNI, with either MMF or sirolimus. An additional validation cohort was also analyzed, consisting of 99 males without rejection and 38 individuals with AR. SNPs for the test cohort were genotyped using the Affymetrix ParaAllele system for SNP detection and genotyping. Additional SNPs and DNA from the validation cohort was genotyped using TaqMan analysis. For statistical analysis, a chi-square test to assess the significance of association of each SNP with AR was used. Haplotype analysis was performed using the R package haplo.stats. By analyzing males only we associated two anonymous X- linked SNPs with AR (rs2189394, $p = 0.008$ and rs2211463, $p = 0.02$). These SNPs were not significant when analyzed in females alone showing that predisposing AR alleles at this locus are acting as X-linked recessive alleles. These markers are near the locus LOC646193, located at Xq13.3. We then analyzed additional SNPs flanking and within this locus. The most significant SNP was rs2301467 with a p value for the test cohort of 1.37×10^{-14}, a p value of 0.002 for the validation cohort and a combined p value of 7.87×10^{-12}. Using the most significant SNPs, there were two major haplotypes identified. The major haplotype gave an odds ratio of 1.8 for AR. Conversely, a second haplotype was protective of AR with an odds ratio of 0.00004. This provides evidence for an X-linked locus associated with AR in kidney allograft recipients.

Integration of structural variants and single nucleotide polymorphisms in linkage and association studies. *B. Kerner[1], P. Sanchez[1], G. K. Chen[2], J. DeYoung[1], M. Almonte[1], O.-W. Choi[1], N. B. Freimer[1]* 1) Department of Psychiatry, UCLA, Los Angeles, CA; 2) Department of Preventive Medicine, USC, Los Angeles, CA.

The telomeric region on Chromosome 8p is a genomic region known for a common inversion/deletion polymorphism. This region has also been claimed for linkage and association with psychotic bipolar disorder and schizophrenia. Inversions and deletions are difficult to detect. However, recent studies have indicated that these regions are characterized by unusually high linkage disequilibrium (LD) patterns among high-density SNPs in individuals who carry the inversion (Bansal V, 2007). This LD pattern can potentially influence the results of linkage and association studies. In our analysis, we applied the following three methods to help infer a polymorphic inversion at 8p23 in families from the NIMH-Bipolar Genetics Initiative, in which evidence of linkage has been found. 1. The pattern of Mendelian errors and failed SNPs during genotyping suggested genomic structure abnormalities in this region. 2. The computer software program GOLDEN HELIX was used to determine the linkage disequilibrium in this genomic region in our data and to explore copy number variations that often flank regions of inversions and deletions. The segmentation algorithm integrated in this software program allowed to determine the segments in each sample separately and to find the cut-points of each segment for each sample. 3. Based on the inferred locations of the flanking regions of the inversion, the posterior probability of carrying an inversion was calculated in each individual of a pedigree using the computer software program MENDEL. We then performed family based linkage and association analysis in these families with psychotic symptoms as phenotype. Taking the deletion/inversion polymorphism into account influenced our results of linkage and association with SNPs in this chromosomal region. Our approach and results demonstrate the importance of integrating information on structural variants with SNP data.

Filamin A Mutations Cause Tetralogy of Fallot (ToF) with Ascending Aortic Aneurysm. *N. D. Patel[1], D. H. Kim[1], M. E. Lindsay[1], T. M. Holm[1], A. John[2], J. Garbarini[2], E. Goldmuntz[2], H. C. Dietz[1]* 1) Institute of Genetic Medicine and HHMI, Johns Hopkins Univ, Baltimore, MD; 2) Children's Hosp Phila, Philadelphia, PA.

ToF is the most common form of complex congenital heart disease. Although surgery during infancy can be curative, a subset of individuals are at risk of developing progressive ascending aortic aneurysm (AAA) years after ideal surgical repair. While there are known etiologies for ToF, the genetic basis for ToF/AAA remains unknown. The absence of large families with this condition precludes positional strategies for gene identification. We have focused on rare patients with syndromic presentations of ToF/AAA. We identified a male patient with a maternal history of periventricular nodular heterotopia (PVNH), a condition caused by mutations in the FLNA gene on chromosome X that encodes the cytoskeletal protein filamin A. Prior reports suggest that male inheritance of a PVNH-associated FLNA allele results in fetal demise. Sequencing of all exons of FLNA revealed two missense substitutions that were hemizygous in the boy with ToF/AAA, heterozygous in his mother and aunt with PVNH, and absent in unaffected relatives and controls. Both mutations substitute evolutionarily conserved residues and one occurs within a domain that interacts with multiple TGF regulatory factors (R-Smads, caveolin and decorin), correlating well with prior data suggesting that multiple forms of aneurysm are caused by perturbation of TGF signaling. We next sequenced FLNA in 11 unrelated patients with nonsyndromic TOF/AAA who had previously been shown not to harbor deletion at 22q11. Two of 11 (18%) had the same missense mutation in the hinge region of the FLNA protein that disrupts a calpain cleavage site. This substitution was not found in >400 control X chromosomes. The conclusion that disruption of filamin A function causes TOF/AAA is furthered by the demonstration of truncus arteriosus (another conotruncal defect) in filamin A-null mice and by the rare occurrence of late-onset aortic dilatation in women with PVNH and related phenotypes. This genetic foundation will allow stratification of risk for AAA in patients with ToF and guide the discovery of molecular mechanisms and therapeutic strategies.

Next Steps in Managing Incidental Findings in Human Subjects Research: From Imaging to Genomics. *S. M. Wolf*[1, 2, 3], *J. K. Paradise*[1, 3] 1) University of Minnesota Law School; 2) University of Minnesota Medical School; 3) University of Minnesota Consortium on Law and Values in Health, Environment & the Life Sciences.

Incidental findings arising during the course of human subjects research are a profound but neglected problem and one of tremendous importance to researchers and Institutional Review Boards because of the potential clinical implications. An incidental finding (IF) is a finding concerning an individual research participant that has potential health or reproductive importance and is discovered in the course of conducting research but is beyond the aims of the study. IFs can range from trivial to life-threatening. Examples of IFs include an unexpected finding of misattributed parentage in genetic research on families and an unexpected allele or pattern of genes discovered during genomic microarray research. This NHGRI-funded project has focused on the ethical, legal, and scientific issues raised by IFs in human subjects research, using empirical research on how researchers and institutions currently address IFs in the both the genetics/genomics context and imaging context (neuroimaging and CT colonography) as a basis for normative recommendations. The multidisciplinary project group of 21 national experts published groundbreaking recommendations in June 2008 for managing IFs in genetics and genomic research in 36(2) Journal of Law, Medicine & Ethics 219-248 (2008), as part of a 17-article symposium. Ours are the first major consensus recommendations on how to handle IFs in human subjects research. This presentation will move beyond the consensus paper to address next steps to handle the growing problem of IFs and suggest how to implement project recommendations. The presentation will offer needed analysis on handling IFs in large genome-wide research protocols and archived datasets. We will address both ethics and law, including the scope of the Common Rule, potential for civil liability, and pragmatic problems such mechanisms to implement the IF recommendations at the level of institutions, IRBs, and researchers.

Collaborative genome-wide association analysis of 10,596 individuals supports a role for Ankyrin-G (*ANK3*) and the alpha-1C subunit of the L-type voltage-gated calcium channel (*CACNA1C*) in bipolar disorder. *P. Sklar[1, 3], M. Ferreira[1, 3], D. Ruderfer[1, 3], J. Smoller[1, 3], H. Gurlin[2], A. McQuillin[2], E. Scolnick[3], A. Corvin[4], D. Blackwood[5], M. O'Donovan[6], M. Owen[6], N. Craddock[6], S. Purcell[1, 3], WTCCC; STEP-BD* 1) Dept Psychiatry, Harvard Medical Sch, Boston, MA; 2) Molecular Psychiatry Laboratory, Department of Mental Health Sciences, Windeyer Institute of Medical Sciences, University College London, 46 Cleveland Street, London W1T 4JF; 3) Stanley Center for Psychiatric Research, Broad Institute of Harvard and MIT; 4) Trinity Centre for Health Sciences, St James's Hospital, Trinity College Dublin, Republic of Ireland; 5) Division of Psychiatry, University of Edinburgh, Royal Edinburgh Hospital, Edinburgh EH10 5HF, UK; 6) Department of Psychological Medicine, Henry Wellcome Building, School of Medicine, Cardiff University, Heath Park, Cardiff CF14 4XN, UK.

To identify susceptibility loci for bipolar disorder, we combined individual genotyping data from the Wellcome Trust Case Control Consortium (WTCCC), Systematic Treatment Enhancement Program for Bipolar Disorder (STEP-BD), University College London (UCL), University of Edinburgh and Trinity College Dublin samples, resulting in 4,387 cases and 6,209 controls. Including imputed single nucleotide polymorphisms, we tested ~1.8 million variants and identified a region of strong association (rs10994336, $P = 9.1 \times 10^{-9}$) in the Ankyrin-G gene, *ANK3*. We also found further support for the previously reported alpha-1C subunit of the L-type voltage-gated calcium channel gene, *CACNA1C* (combined $P = 7.0 \times 10^{-8}$, rs1006737). Taken together, our results underscore the value of large, collaborative studies and suggest that ion channelopathies may be involved in the pathogenesis of this complex disorder.

-383 A/C Tumor Necrosis Factor Receptor 1 polymorphism and serum levels of TNFR I and TNFRII in rheumatoid arthritis. *Y. Valle[1,2], I. Ledesma-Lozano[1], N. M. Torres-Carrillo[1], N. Torres-Carrillo[1], J. R. Padilla-Gutiérrez[1,2], R. E. Navarro-Hernández[1], M. Vázquez-Del Mercado[1], E. Sánchez-Corona[1], J. Armendáriz-Borunda[3], J. F. Muñoz-Valle[1]* 1) IIRSME, C.U.C.S, UdeG; 2) Posdoctoral Fellow in Biomedical Sciences (Immunology), UdeG; 3) Instituto de Biología Molecular y Terapia Génica, C.U.C.S,UdeG.

Introduction . Tumor necrosis factor-alpha (TNFalpha) plays a central role in inflammation, and has been directly implicated in the pathogenesis of rheumatoid arthritis (RA). TNF mediates its pleiotropic activities via two cell surface receptors, p55TNFR (TNFRI) and p75TNFR (TNFRII); both are active in membrane-bound and soluble forms. Soluble receptors act as physiological attenuators of TNF alpha activity. Methods: We recruited 190 RA patients classified according to ACR criteria and 190 healthy subjects (HS). The -383 A/C TNFR1 polymorphism was identified by PCR-RFLP method. The sTNFRI and sTNFRII were measured using a ELISA kit. The clinical activity in RA patients was evaluated using the Disease Activity Score (DAS28). The statistical analysis was made using SPSS v 10.0 Software. Results. The -383 A/C TNFRI polymorphism not showed significant differences in both studied groups. However, in RA patients the AA genotype carriers had the higher DAS28 score (p=0.01). The serum levels of TNFRI and TNFRII were higher in RA patients than HS (p=0.04 and 0.001, respectively). In addition, serum sTNFRI levels correlated with sTNFRII (r=0.699, p=0.000), whereas the sTNFRII correlated with DAS28 (r=0.699, p=0.000), RF (r=0.505, p=0.004) and, ESR (r=0.323, p=0.042). Conclussion. The -383 A/C TNFRI polymorphism is not a susceptibility marker in RA. However, the A/A genotype is associated with a high DAS28 score. The increase of TNFRII serum levels reflect the clinical activity in RA patients because correlated with the DAS28 index, RF and ESR.

Replicated statistical associations between schizophrenia and intronic variations flank a novel cassette exon in the dopamine transporter gene. *M. E. Talkowski[1,5], L. McClain[1], M. Bamne[1], J. Wood[1], M. Chen[2], K. McCan[2], P. Papasaikas[2], G. Kirov[2], L. Georgieva[2], M. C. O'Donovan[3], M. Owen[3], D. Toncheva[4], D. Lewis[1], B. Devlin[1,5], A. J. Lopez[2], V. L. Nimgaonkar[1,5]* 1) Psychiatry, University of Pittsburgh; 2) Biological Sciences, Carnegie Mellon University; 3) Psychological Medicine, Cardiff University; 4) Medical Genetics, University Hospital Maichin Dom, Bulgaria; 5) Human Genetics, University of Pittsburgh.

The dopamine transporter (*SLC6A3*, DAT) is a critical gene involved in the re-uptake of dopamine from the synapse. We previously identified significant, replicable statistical associations and gene-gene interactions between intronic DAT variations and schizophrenia in two large Caucasian samples (Talkowski *et al.*, 2008). We describe a parallel, comprehensive effort to identify plausible common liability loci (minor allele > 5%) in these intronic regions (introns 3 and 4) along with a functional basis for the statistical results. We conducted in-house sequencing using CEPH individuals in conjunction with publicly available resources (HapMap and SeattleSNPs). We genotyped 88 tag SNPs chosen at a high correlation threshold ($r^2 < 0.95$ between loci) to represent most common variations in two independent samples (659 Bulgarian trios and 526 cases / 501 controls from the US). We detected seven significant associations ($p < 0.05$) and nine non-significant trends, 56.3% of which were localized to introns 3/4. These plausible risk loci included multiple SNPs not represented in our studies prior to this fine mapping effort. We found that several SNPs in complete LD with our strongest associations (including a novel deletion) flanked a potential cassette exon. Consistent with sequence predictions, a novel cassette exon within intron 3 of DAT was detected. The cassette exon truncates the DAT protein, is expressed in adult human brain, is conserved among primates, and was confirmed through in vitro studies (see Chen *et al*. abstract for details). Parallel statistical and functional studies are ongoing to further characterize the role of putative schizophrenia risk alleles on dopamine transporter function.

Semaphorins as Candidate Genes in Hirschsprung Disease. *S. Arnold[1], M. Guy[1], K. Kashuk[1], Y. Li[2], G. Abecasis[2], A. Chakravarti[1], International HSCR Consortium* 1) IGM, Johns Hopkins Univ Sch Med, Baltimore, MD; 2) Ctr for Stat Gen, Dept of Biostat, Sch of Pub Health, Univ of MI, Ann Arbor, MI.

Hirschsprung Disease (HSCR) is a complex disorder for which a growing number of candidate genes and chromosomal regions, identified via linkage and various mouse model studies, hypothetically modify the function of the major HSCR gene, the receptor tyrosine kinase *RET*. HSCR varies in the length of colon affected by aganglionosis, from the least severe and most common short segment disease (S-HSCR) to the least common and most severe form, total colonic agangionosis. In order to identify candidate genes that might contribute to the majority of HSCR cases, 220 S-HSCR trios were analyzed on the Affymetrix 500K SNP array platform. A significant cluster of SNPs was identified, via TDT analysis, in a region on Chr. 7 that fell downstream from *SEMA3D* and upstream from additional SEMA Family 3 members (3A, 3E, and 3C, in order of proximity). Imputation, which added ~2 x 10^6 SNPs to the analysis, strengthened the significance of this cluster and refined the location of its peak. The two pre-imputation SNPs with highest significance (SNP1, with p-value of 6.11 x 10^{-6} and SNP2, with p-value of 5.16 x 10^{-6}) were genotyped in an independent replication population of 430 HSCR trios that represented all segment lengths. Both SNPs maintained significance (p-values of 1.39 x 10^{-2} for SNP1 and 2.56 x 10^{-4} for SNP2) in the full replication population. When data obtained from the S-HSCR replication subset was combined with the original Affy data, SNP1 improved to a p-value of 1.57 x 10^{-7} while SNP2 improved to 4.26 x 10^{-6}. These SNPs displayed greater penetrance in males and for individuals homozygous for the transmitted allele, analogous to the *RET+3* enhancer variant. The four SEMA Family 3 members demonstrated very similar temporal and spatial patterns of expression throughout the gut and were coexpressed with *RET* in these tissues, supporting the possibility that one or all might modify *RET* function in the developing enteric nervous system. Consequently, sequencing of coding regions is now underway to determine whether one SEMA Family 3 member can be distinguished as the HSCR-associated gene.

Functional analysis of DUSP2 in Zebrafish in attempt to better understand the molecular mechanisms in Apert Syndrome. *E. Kague[1], S. Fisher[2], R. D. Fanganiello[1], M. R. Passos-Bueno[1]* 1) Dept Genetics & Evolutive Biol, Univ de Sao Paulo, Sao Paulo, Brazil; 2) Johns Hopkins University School of Medicine- Baltimore USA.

Apert syndrome is an autossomal dominant disorder characterized by craniossinostosis and malformations of limbs. It is caused by a limited number of mutations in FGFR2 gene. Two mutations were identified so far, Ser252Trp and Pro253Arg. Mutant mouse carrying the S252W mutation has, besides defect in closure of sutures, skeletal system abnormalities including increase cartilage of the basicranium, malformation of the palate and thickened nasal cartilage. In study, performed in Brazil, attempting to understand gene expression profile of periosteal cells from Apert patients, DUSP2 was found as upregulated in patients (Fanganiello et al., 2008), pointing this gene as a downstream target of FGF signal in the cell and also a target of study for the molecular mechanism involved in Apert syndrome. DUSP2 localizes to the nucleus and inactivates MAPK (JNK) by dephosphorylation. JNK is downstream of Dsh-mediated noncanonical Wnt pathway. To better understand the functionality of DUSP2, we overexpressed it in double transgenic Zebrafish embryos (carrying EGFP and mChrerry as cartilage and bone markers, respectively). It was possible to classify injected embryos in two groups. Group one embryos showed phenotype similar with Silberblick (wnt11/slb) mutants, with incomplete separation of the eyes, what could be explained by over inhibition of JNK by DUSP2. Class two embryos had no eyes commitment but just showed cartilage malformation including defects in Meckels cartilage, palatoquadrate, ceratohyal and ceratobranchials, representing its importance in bone formation. We carried out dusp2 in situ hybridization in zebrafish, and it showed a restricted expression, and quite similarity with fgfr2. At 90% epiboly dusp2 is expressed in paraxial mesoderm, at 8 somites in eyes field, forebrain and midbrain, at 24h and 48h in forebrain and midbrain. The results reinforce DUSP2 importance in bone formation, pointing it as a potential target in the molecular mechanism of Apert Syndrome and it also indicates participation of non-canonical wnt signaling, but further studies must be performed.

Genomic Landscape of Autism Spectrum Disorders. *M. Bucan[1, 2], K. Wang[2, 5], J. Glessner[5], M. Imielinski[5], D. Hadley[2, 3], J. Bradfield[5], C. Kim[5], L. Sonnenblick[7], N. Gidaya[2], V. Kustanovich[1], A. Singelton[6], C. Lajonchere[1], J. Kim[3], M. Li[4], R. Cantor[1,8], B. Abrahams[7], S. Grant[5], D. Geschwind[1, 7,8], H. Hakonarson[5]* 1) Autism Genetic Resource Exchange, Autism Speaks; 2) University of Pennsylvania, Department of Genetics; 3) University of Pennsylvania, Department of Biology; 4) University of Pennsylvania, Department of Biostatistics; 5) Center for Applied Genomics6, Division of Genetics7, The Children's Hospital of Philadelphia; 6) Porter Neuroscience Research Center, National Institute of Aging, National Institute of Health; 7) Department of Human Neurology, University of California Los Angeles; 8) Department of Human Genetics, University of California Los Angeles.

Genomic copy number variants (CNVs) are important to the etiology of complex diseases such as the autism spectrum disorders (ASDs). However, published work suggests that identified CNVs represent a relatively small number of disease-linked variants. We have undertaken a comprehensive CNV analysis to assess the contribution of CNVs in the ASDs. With the HumanHap550 BeadChip containing 561,466 SNPs, we genotyped 943 autism multiplex families (4,454 individuals) from the Autism Genetics Resource Exchange (AGRE) and 1,612 control subjects from two independent groups. Using the PennCNV algorithm, we detected ~164,000 CNVs and classified them as common or rare, and inherited or de novo. De novo CNV >200kb were identified in 3.0% of ASD subjects versus 2.4% of their unaffected siblings. Remarkably, rare CNVs surrounding or overlapping 37 membrane-spanning neuronal genes, including CDH18, CNTNAP2, NRXN1, NCAM2 and SLITRK1, were enriched in cases versus unaffected siblings and unrelated controls (P=1.9e-4). These data indicate that previous work captured only a small subset of CNVs likely to be important in disease pathogenesis. Our results also suggest that rare CNVs, both inherited and de novo, particularly in genes encoding neuronal membrane proteins, contribute to the pathogenesis of autism. The work described here will serve as an important foundation for the study of genomic aspects of diseases.

A Follow-Up of Enzyme Replacement Therapy in Two MPS VI Patients with Poorly Engrafted Bone Marrow Transplantation. *V. Valayannopoulos[1], M. Raff[2], S. Turbeville[3], H. Nicely[3]* 1) Metabolic Unit, Necker-Enfants Malades Hosp, Paris, France; 2) Children's Hospital and Regional Medical Center, Seattle, WA, USA; 3) BioMarin Pharmaceutical Inc., Novato, CA, USA.

Background & Purpose: Mucopolysaccharidosis VI (MPS VI) is a genetic lysosomal storage disorder classified by missing or defective enzyme *N*-acetylgalactosamine-4-sulfatase and accumulation of glycosaminoglycan (GAG) dermatan sulfate. In some cases, HLA-matched bone marrow transplantation (BMT) has been an appropriate treatment option. With approval of Naglazyme (galsulfase) in 2005 (US) and 2006 (EU), enzyme replacement therapy (ERT) became available to MPS VI patients including poorly-engrafted BMT patients, 2 of whom were followed using a BioMarin-designed questionnaire completed by 2 physician authors. **Method**: Weekly ERT infusions of Naglazyme at 1mg /kg over a minimum of 4 hrs were given to 2 rapidly progressing MPS VI patients with severe symptoms: Patient A (female; 4 y; U.S.) and Patient B (male; 18 y; France), respectively; both with increased urinary GAG levels, decreasing chimerism over time and disease progression on BMT, prior to ERT. Physicians recorded clinical changes post-ERT initiation on a questionnaire. **Results**: Patient A at 1 y 7 mo after first ERT showed improvements in visual acuity, cardiac function, endurance, general health; with a non-significant reduction in urinary GAGs from 99.7 g/mg creatinine pre-ERT to 86.0 g/mg creatinine at 3 mo on ERT; and improvement in subcortical and periventricular white matter. Infusion-associated reactions (IAR) included arthralgia, fever and headache, which were safely resolved and this patient continues to be treated with ERT. Patient B by 9 mo of ERT, showed improvements in dysmorphia, visual acuity, joint flexibility, ear, nose and throat dysfunctions; with reduced urinary GAGs from 51.8 g/mmol creatinine pre-ERT to 19 g/mmol creatinine at 9 mo on ERT; and no reported IARs. Unfortunately, Patient B died suddenly due to underlying cardiopathologies unrelated to ERT at 9 mo on drug. **Conclusion**: Administration of Naglazyme ERT after poorly engrafted BMT was well tolerated and provided some clinical benefit to 2 MPS VI patients.

Functional and predictive analysis of variants of the Wilson Disease Gene, *ATP7B*. *L. Luoma, T. M. M. Deeb, G. Macintyre, D. W. Cox* Department of Medical Genetics, University of Alberta, Edmonton, Canada T6G 2H7.

Wilson disease (WD), an autosomal recessive disorder with an incidence of approximately 1 in 30,000 worldwide, affects copper homeostasis. Copper is essential for structure and catalytic activity of many enzymes, however due to its reactivity can be toxic to cells at high concentrations. WD is characterized by excess copper in the liver, kidney and brain leading to hepatic, renal and/or neurological damage. Because of the wide range of manifestations, WD is challenging to diagnose, but treatable. Since discovery of the causative gene, *ATP7B*, a copper-transporting ATPase that has a critical role in copper homeostasis, WD can be diagnosed by mutation analysis. Mutation of *ATP7B* affects its ability to transfer copper to other proteins and prevents excretion of copper, resulting in copper overload and cell damage. *ATP7B* variants, both disease-causing and normal, occur throughout the gene, but are difficult to distinguish. An effective way to determine if a variant is disease-causing is to do functional analysis, using yeast and mammalian model systems. However, this testing is labor- and time-intensive and we have explored the value of predictive programs. Computer-based predictive programs such as SIFT (Sorting Intolerant from Tolerant), Align GVGD and PolyPhen may be helpful for determining whether a newly identified variant is deleterious, or functionally normal. We tested accuracy of predictive programs by comparison of predictions with functional analysis in yeast assays of the same variants. We investigated 31 variants identified in WD patients located in the ATP-loop and transmembrane (TM) regions of ATP7B. Of the three programs PolyPhen appears to be best at predicting disease variants. SIFT could be adjusted to be as accurate by modifying sequences used. Of 18 variants in the TM domains that were functionally tested in yeast, 15 were found to be deleterious. Predictions by PolyPhen and adjusted-SIFT were 83% accurate. Accuracy of predictions was found to be dependent on sequences used in the analysis. Use of both functional studies and predictive programs may lead to a better understanding of *ATP7B* and assist in reliable DNA diagnosis of WD.

The mutational spectrum of BCOR: Candidate gene analysis of patients with oculofaciocardiodental and Lenz microphthalmia syndromes, mental retardation and ocular anomalies, and cardiac laterality defects. *S. Whalen[1], E. Hilton[2], J. Johnston[3], N. Okamoto[4], Y. Hatsukawa[4], L. Biesecker[3], I. Giurgea[1], G. Black[2]* 1) INSERM 654, Department of Genetics & APHP, Hospital Henri Mondor, Creteil, France; 2) Academic Unit of Medical Genetics, St Mary's Hospital, Manchester, UK; 3) Genetic Disease Research Branch, National Human Genome Research Institute, NIH, Bethesda, MD, USA; 4) Maternal and Child Health, Osaka Medical Centre and Research Institute, Osaka, Japan.

OFCD and Lenz microphthalmia syndromes form part of a spectrum of X-linked microphthalmia disorders characterised by ocular defects, dental anomalies, cardiac defects, digital and skeletal anomalies and mental retardation. The two syndromes are allelic and caused by mutations in the BCL-6 corepressor (BCOR). To extend the series of phenotypes associated with pathogenic mutations in BCOR, we have sequenced the BCOR gene in patients with 1) OFCD syndrome 2) putative X-linked (Lenz) microphthalmia syndrome 3) isolated ocular defects and 4) laterality phenotypes. We present a new cohort of females with OFCD syndrome and null mutations in BCOR, supporting the hypothesis that BCOR is the sole molecular cause of this syndrome. The identification of a BCOR mutation in a female with ocular defects in the absence of a classic OFCD syndrome phenotype demonstrates the phenotypic variation observed in this syndrome and suggests that OFCD syndrome may be underdiagnosed. We have sequenced a cohort of males diagnosed with putative X-linked (Lenz) microphthalmia and found a mutation in a single case, suggesting that BCOR mutations are not a major cause of X-linked microphthalmia in males. The absence of BCOR mutations in a panel of patients with non-specific laterality defects suggests that mutations in this gene are not a major cause of isolated heart and laterality defects. Analysis of the phenotypes associated with OFCD and Lenz microphthalmia syndromes shows that in addition to the standard diagnostic criteria of congenital cataract, microphthalmia and radiculomegaly, patients should be examined for skeletal defects, particularly radioulnar synostosis and cardiac/laterality defects.

Chromosome abnormalities in spontaneous abortions: a cytogenetic study of 4068 cases. *L. Dong, W. Bai, R. Habibian, J. Wang, B. Huang, A. Hajianpour* Cytogenetics Lab, Genzyme Genetics, Monrovia, CA.

It is well known that about 10-15% of recognized pregnancies result in spontaneous abortion (SA) with chromosomal abnormalities being the most frequent cause of SA. Maternal age is an important factor and a leading contributor to chromosomal abnormalities in SA cases. We retrospectively reviewed the cytogenetic results from our laboratory for specimens of products of conception (POC) with the clinical indication of SA between 2005 to the present. A total of 5545 POC specimens were received for chromosome analysis. After careful examination, 4803 (86.6%) cases with recognized villi and/or fetal tissues were cultured for cytogenetic evaluation. We obtained cytogenetic results for 4068 (84.7%) cases, of which, the sex ratio (XY/XX) was 1.12. Chromosome abnormalities were observed in 2461 (60.1%) cases. The most frequent abnormalities detected were autosomal trisomies (1567/2461, 63.7%), followed by triploidy or near-triploidy (282 cases, 11.5%), 45,X (226 cases, 9.2%), unbalanced structural rearrangements (116 cases, 4.7%), double trisomies (78 cases, 3.2%), tetraploidy or near-tetraploidy (74 cases, 3.0%), balanced rearrangements (31 cases, 1.3%) and monosomy 21 (23 cases, 0.9%). Of note, monosomy 21 was the only autosomal monosomy we observed. Trisomy was identified for all autosomes except of chromosome 1. In 1567 trisomy cases, the most common trisomy was chromosome 16 (385 cases, 24.6%), followed by chromosomes 22 (268 cases, 17.1%), 15 (188 cases, 12.0%), 21 (136 cases, 8.7%), 13 (97 cases, 6.2%) and 18 (72 cases, 4.6%). The single most common anomaly identified in our study was trisomy 16 (385/2461, 15.6%). In cases with unbalanced structural rearrangements, 32 (27.6%) were due to Robertsonian translocations. We also evaluated the relationship between maternal age and chromosome abnormalities in SA. The median maternal age for the abnormal chromosome group (35.4 +/- 5.5 years) was higher than that for the normal chromosome group (32.9 +/- 6.1 years). The difference was statistically significant at $P < 0.0001$. In conclusion the cytogenetic results obtained in spontaneous abortions in our laboratory were similar to those reported in the literature.

Sequencing a female genome in an academic hospital - what did we learn? *Y. Ariyurek[1], M. Kriek[1], M. Van Galen[1], P. De Knijff[2], M. P. Villerius[1], M. H. Breuning[1], G. J. B. Van Ommen[1], J. T. Den Dunnen[1]* 1) LGTC-Human & Clinical Genetics, Leiden University Medical Center, Leiden, Netherlands; 2) FLDO-Human & Clinical Genetics, Leiden University Medical Center, Leiden, Netherlands.

Current developments in DNA sequencing technology go very fast. Although the industrial goal to sequence a human genome for € 1000 has not yet been achieved, it will be possible soon. For any academic hospital the possibility to sequence a complete genome in order to resolve a health problem that might have a genetic cause seems an attractive option. To experience what hurdles we will meet, technically, computationally and analytically, we sequenced the genome of a European female, a clinical geneticist. From this we learned that technologically, even in an academic hospital setting, it is already feasible. It only costs time (a few months) and money (50-100K Euro). Computationally it was at the limits of our possibilities; with data storage and data transfer negatively influencing the final full sequence assembly and interpretation. The analysis of the genome shows it is female, that we can follow its ancestry (mainly European, based on mitochondrial and genomic DNA), that we can predict some of her appearance (e.g. red hair, blue eyes), that it contains no major early-onset genetic disease mutations and that it carries varying risks for all kinds of other traits. As expected, the genome analysis was beyond our current capabilities. Partly this relates to the computational hurdles mentioned, but the key problem was the analysis of all the variants we found compared to the human genome reference sequence (> 1 million). There is currently no software to score these variants effectively and existing information on sequence variants is limited and scattered over many databases, websites and diverse publications. In fact this is the reason why sequencing a patient's genome, although technically possible, cannot be applied on a routine basis to answer health related issues. To resolve this problem we need to develop mechanisms to efficiently collect and share all sequence variants identified, with their phenotypic consequences, and to connect this information to the reference genome sequence.

Fibroblast Growth Factor Receptor Gene Polymorphisms: Association with Obstructive Sleep Apnea in Children. *M. Kalra[1], P. Pal[3], L. Dolan[2], J. Mallik[3], S. Guha[3], R. Deka[3], R. Chakraborty[3]* 1) Dept Pulmonary Medicine, Cincinnati Children's Hosp, Cincinnati, OH; 2) Dept Endocrinology, Cincinnati Children's Hosp, Cincinnati, OH; 3) Center for Genome Information, University of Cincinnati.

Obstructive sleep apnea (OSA), which affects 2%-5% children, is associated with neurocognitive deficits and cardiovascular and metabolic sequelae. Although genetic predisposition for OSA has been demonstrated, the exact genetic determinants are not yet known. With strong evidence for craniofacial characteristics as its risk factors, genes that are involved in craniofacial developments such as Fibroblast Growth Factor Receptor 2 (FGFR2) have been proposed as candidate genes for OSA. The objective of this study was to test the association of FGFR2 polymorphisms and OSA status. All Caucasian children diagnosed with OSA at Cincinnati Childrens Sleep Center between January and June 2006 were recruited as cases (n = 165) and ethnicity matched controls (n= 381) were selected from the population-based Princeton School District Study. Four SNPs (rs1649161, rs2981430, rs2981452, and rs2420946) that tag the FGFR2 gene were selected using SNPbrowser ver. 3.5 (ABI). Genotyping was performed using the SNPlex (ABI) high-throughput genotyping platform. OSA was defined as apnea hypopnea index >1 on polysomnogram; cases were compared to population-based controls. The mean age of the OSA cases was 11.5 years (S.D. 4.4), 58% were males, and mean BMI was 30.7 (S.D. 12.0). The mean age of the controls was 14.6 years (S.D. 2.1), 55 % were males, and mean BMI was 22.9 (S.D. 3.4). Structure analysis revealed absence of significant population stratification between cases and controls. Allele frequency differences between cases and controls were significant for 2 SNPs (rs2981452, $p< 0.01$; and rs2420946, $p< 0.01$). Multivariate logistic regression analysis with BMIz score as covariate revealed independent genotypic association at SNP rs2420946 with OSA status (OR 5.0, 95 %CI 2.0-10.0). This is the first report on association of FGFR2 polymorphisms with OSA in children, supporting the role of FGFR2 in the pathogenesis of pediatric OSA.

Leveraging regulatory features to prioritize SNP markers for follow-up studies. *M. A. Levenstien, R. J. Klein*
Cancer Biology and Genetics, Memorial Sloan-Kettering Cancer Center, New York, NY.

Curation of the human genome has expanded to include regulatory features based on experimental evidence from genome-wide assays. This information represents a powerful resource for prioritizing regions of the genome outside of annotated coding regions as having functional importance. We target these regulatory features as a means to identify and prioritize groups of SNPs that are more likely to affect phenotypes in order to facilitate efficient SNP selection for follow-up studies to genome-wide tests of association where signals exist far from an annotated protein-coding region. Based on the annotations available in the Ensembl database, we categorize SNPs in the human genome into classes related to regulatory features, such as epigenetic modifications and factor binding sites. Using the distribution of derived allele frequencies (DAF) within each class, we assess the strength of natural selection for each class relative to the genome as a whole. We apply this DAF analysis to Perlegen resequenced SNPs genome-wide. Groups of regulatory elements annotated by Ensmbl as well as some individual elements, such as PolII binding sites, DNase I hypersensitivity sites, and several histone methylation sites, show negative selection in comparison to the genome as a whole. In fact, some histone modification sites show derived allele frequencies comparable with those of the coding regions. These DAF distribution comparisons highlight which functional classes are under negative selection, have functional importance, and contain SNPs which are strong candidates for follow-up studies.

Facioscapulohumeral muscular dystrophy: gene discovery by DNase-chip. *M. Ehrlich[1], X. Xu[1], K. Tsumagari[1], G. Crawford[2]* 1) Human Genetics, Tulane Med. Sch., New Orleans, LA; 2) Inst. for Genome Sciences & Policy, Duke Univ., Durham, NC.

Facioscapulohumeral muscular dystrophy (FSHD) patients have one short array (<11 units) of tandem 3.3-kb repeats (D4Z4) about 15-25 kb from the end of 4q (4q35.2). Nonpathogenic D4Z4 arrays contain 11-100 units. There are almost identical arrays near the end of 10q (10q26). Short 10q arrays are phenotypically neutral despite high 4q/10q homology 42 kb proximal to D4Z4 and in all distal sequences; similar size polymorphisms; and a common candidate FSHD gene, *DUX4*, in their 3.3-kb repeats. There should be a disease-related sequence in 4q35.2 that is responsible for the pathogenicity of short D4Z4 arrays only at 4q. No 4q35 sequence differentially expressed in FSHD *vs.* control muscle was seen in several microarray expression analyses. To discover undocumented 4q35.2 genes possibly related to FSHD, including noncoding RNA genes, we looked for DNaseI hypersensitive (DH) sites, a gene hallmark. By DNase-chip analysis with high-resolution tiling arrays, DH sites were examined in the terminal 4 Mb of 4q in three FSHD and three control myoblast cell populations. The proximal 1.5 Mb of 4q35.2 had a normal density of DH sites and suggested the presence of undocumented genes. In the most distal 2.5 Mb of 4q, only one DH peak was seen in all myoblast cell strains that was not attributable to short tandem repeats. It overlapped the 5 end of *FRG1*, a gene which did not show FSHD-specific expression. FSHD myoblasts preferentially displayed one DH site 272 kb proximal to 4q D4Z4. Nearby, we found a transcribed sequence with homology to a predicted noncoding RNA. The very low density of DH sites in the terminal 2.5 Mb of 4q is consistent with the very low percentage of sequences overlapping RefSeq genes or UCSC gene predictions. In contrast, there was a normal density of DH sites at the end of 10q.These results suggest that a unique sequence about 0.3 Mb proximal to D4Z4 might determine the FSHD linkage of short D4Z4 arrays to 4q, but not to 10q. Alternatively, our data support the hypothesis that the extreme paucity of genes at 4q35.2 contributes to the 4q-specific nature of the disease (Supported in part by NIH Grant NS048859 and FSH Society Grant FSHS-MGBF-013).

A Novel Mutation in the NTRK1 Gene Causes Congenital Insensitivity to Pain and Anhidrosis among Moroccan Jews. *T. Falik-Zaccai[1,2], C. Suriano[1], A. Zinger[3], N. Kfir[1], M. Weiler[1], C. Aslanidis[4], G. Schmitz[4], M. Khayat[1]* 1) Inst. Human Gen, Western Galilee Hosp, Nahariya, Israel; 2) Rappaport Faculty of Medicine, Technion, Haifa, Israel; 3) Genetics Institute, Barzilai Medical Center, Ashkelon, Israel; 4) Institute for Clinical Chemistry and Laboratory Medicine, University of Regensburg, Medical Faculty, Regensburg, Germany.

Congenital insensitivity to pain with anhidrosis (CIPA) or Hereditary sensory and autonomic neuropathy type IV (HSAN IV) is a rare, autosomal recessive (AR) neurologic disorder, characterized by absence of reaction to painful stimuli, mental retardation, self- mutilating behavior, anhidrosis, and recurrent episodes of hyperthermia. 48 mutations in the neurotrophic tyrosine kinase receptor 1 (NTRK1), a receptor phosphorylated by nerve growth factor (NGF), were identified. Approximately 100 patients are described in the literature worldwide,from diverse ethnic groups, including five of Moroccan Jewish origin. The prevalence of CIPA mutations, and the molecular basis of the disease in this small homogenous ethnic group, has not been previously described. We identified a novel nonsense mutation in two unrelated families of Moroccan Jewish descent, each with two affected siblings. Haplotype analysis revealed a possible founder mutation that may trace to a rural Jewish village in the Atlas Mountains. Genetic screening for the causative mutation among 300 unrelated Moroccan Jews did not reveal any carriers for the causative mutation, thus excluding high risk for CIPA in this ethnic sub- population.

Intragenic deletions of the *CDKL5* gene in female patients with infantile spasms and atypical Rett syndrome: A pilot study. *S. C. Reshmi, A. J. Whited, R. J. Alva, E. V. Haverfield, M. Dempsey, S. Das* University of Chicago, Department of Human Genetics, Chicago, IL.

Atypical Rett syndrome is a neurodevelopmental disorder associated with early-onset epileptic seizures and infantile spasms. The atypical Rett syndrome phenotype ranges from milder forms of the classic Rett syndrome with mild mental retardation and slower progression, to more severe forms that manifest at birth. Approximately 20% of patients with atypical Rett syndrome have mutations in the *MECP2* gene. Of patients in whom no mutation has been identified, approximately 3% have partial deletions in *MECP2*. Mutations in either *CDKL5* or *ARX*, both also mapping to the X chromosome, appear to have some phenotypic overlap with the severe form of atypical Rett syndrome with infantile spasms. We studied a series of female patients referred to our diagnostic laboratory for atypical Rett syndrome and/or infantile spasms and in whom no mutations were identified by *CDKL5* or *ARX* sequence analyses, for intragenic deletions and duplications of the *CDKL5* and *ARX* genes. Of 97 patients analyzed thus far, we have identified three individuals (3.1%) with intragenic deletions in exon 1 of the *CDKL5* gene. These deletions were detected by MLPA analysis and confirmed by real-time quantitative PCR. Exon 1 of the *CDKL5* gene is a non-coding exon and is located within the promoter region of the main *CDKL5* isoform 1. To date, deletions of this exon have not been reported in patients with atypical Rett syndrome/infantile spasms nor have they been described as variants/polymorphisms of the *CDKL5* gene. One of the original *CDKL5*-positive patients described with infantile spasms had an apparently balanced translocation involving the X chromosome. The breakpoint occurred in intron 1 of the *CDKL5* gene, resulting in separation of exon 1 from the coding region of the gene and absent gene expression (Kalscheuer et al., 2003). We speculate that a deletion in exon 1 of the *CDKL5* gene may also likely affect its normal expression and result in disease. We are in the process of further clarifying our exon 1 deletion finding to better determine its role in atypical Rett syndrome and infantile spasms.

An Overview of Molecular Abnormalities and Genotype-Phenotype Correlation in Cornelia de Lange Syndrome: Perspectives from the Molecular Diagnostic Laboratory. *E. V. Haverfield, A. J. Whited, R. J. Alva, L. Blumenfeld-Kouchner, A. I. Lozada, A. J. Reeder, L. L. Wysinger, M. A. Dempsey, S. Das* Department of Human Genetics, University of Chicago, Chicago, IL.

Cornelia de Lange syndrome (CdLS) is a rare developmental disorder characterized by distinctive facial features, growth retardation, hirsutism, and upper limb reduction defects. Mutations in the *NIPBL* and *SMC1A* genes have been identified in patients with CdLS with a frequency of ~50% and 3-5%, respectively. We have analyzed over 250 patients for abnormalities that cause CdLS through *NIPBL* and *SMC1A* full gene sequence analysis, as well as *NIPBL* deletion/duplication analysis by multiplex ligation-dependent probe amplification (MLPA), in our molecular diagnostic laboratory. We have identified over 40 novel pathogenic mutations in the *NIPBL* gene by DNA sequencing in 210 patients analyzed (23.8%). *NIPBL* deletion/duplication analysis identified 3 intragenic deletions and 1 intragenic duplication in 124 individuals analyzed, a detection rate of 3.2%. The *NIPBL* intragenic duplication, a mutation type that has not been described to date, was detected in a newborn female with a classic CdLS phenotype. The overall mutation detection rate in our patient cohort that was analyzed by both DNA sequencing and MLPA was 54.6% for mutations and deletions/duplications in the *NIPBL* gene. The rate of mutation detection in the *SMC1A* gene has been low and has been observed in 1 out of 79 patients analyzed (1.3%). Detailed clinical information on all patients analyzed for *NIPBL* and *SMC1A* is being collected via a questionnaire that is submitted to our laboratory with the patient sample at the time of test request. We will present the clinical findings in patients with and without abnormalities in the *NIPBL* and *SMC1A* genes. Our findings represent an update on the molecular basis of CdLS as well as provide a genotype-phenotype correlation of *NIPBL* and *SMC1A* mutations and disease severity. Our studies also illustrate that *NIPBL* intragenic deletions and duplications are a contributor to CdLS etiology.

Identification and characterization of NIPBL physiological splicing variants. *F. J. Ramos[1], M. Ciero[2], B. Puisac[2], M. C. Gil-Rodríguez[2], M. Arnedo[2], M. P. Ribate[2], J. C. de Karam[2], M. Ramos[2], S. Menao[2], A. Pié[2], J. Pié[2]* 1) Secc. Genética, Depto. Pediatría, Fac. Medicina, Universidad de Zaragoza, Zaragoza, Spain; 2) Laboratorio de Genética Clínica y Genómica Funcional. Depto. Farmacología-Fisiología, Fac. Medicina, Universidad de Zaragoza, Zaragoza, Spain.

INTRODUCTION: Cornelia de Lange Syndrome (CdLS) is a clinically heterogeneous inherited disorder characterized by distinctive dysmorphic facial features, growth and cognitive impairment, and limb malformations. In 2004, two independent groups, Krantz et al. and Tonkin et al., found mutations in a regulator of the cohesin complex, the NIPBL gene, located in 5p13.2, in patients with Cornelia de Lange Syndrome 1 (CDLS1, MIM 122470). NIPBL contains 47 exons and predicted to generate two isoforms. Northern-blot and in situ analyses showed that NIPBL was expressed in several tissues with different level of expression.The identification of multiple transcripts in this gene suggest the presence of alternative splicing, a feature that has not been yet studied. This is the first systematic analysis of the NIPBL splicing variants in human normal tissues. AIM OF THE STUDY: To determine the existence of alternative splicing in NIPBL gene. METHODS: We have amplified the NIPBL cDNA from fetal skeletal muscle, fetal brain, adult leukocytes and adult brain, in various overlapping fragments. PCR products were separated by electrophoresis on agarose gel. Additional bands of different length than expected were sequenced. RESULTS: Seven different splicing variants were found and all deletions observed were in frame. Some of them were expressed only in one tissue like NIPBL-43, NIPBL-45 and NIPBL-[E10,E12]. In contrast, NIPBL-12 and NIPBL-33/34 are present in all studied tissues. To investigate the significance of these variants we are quantifying them by real time PCR in different tissues. Preliminary studies have shown a low expression level of some transcripts. Although their functional significance is still unclear, we hypothesize that some splicing variants might have a biological role. *This work is supported by a grant from the Ministerio de Sanidad of Spain (Ref. PI061343) and the Diputación General de Aragón (Ref. B20).* .

Further support for the involvement of ASAH2L in Alzheimers disease. *R. Wang[1], S. S. Bassett[1], D. Avramopoulos[1,2]* 1) Psychiatry, Johns Hopkins University School of Medicine, Baltimore, MD; 2) McKusick Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD.

We have previously reported the identification of a new gene, ASAH2L, which showed evidence for involvement in the risk to develop Alzheimers Disease (AD). ASAH2L is created by a partial duplication of the ASAH2 gene, an event only observed in the human genome. It is located in a region strongly linked to AD and we previously showed that it has an expression profile consistent with a correlation of higher transcription with protection against neurodegeneration. We have now acquired an independent set of temporal lobe brain samples and replicated the expression results with stronger significance than the original study. Further, in order to explore whether this transcript is indeed translated into protein we sought to determine whether any of the two putative start codons present in the ASAH2L transcript can be used to initiate the translation of their corresponding non-overlapping open reading frames (ORF1 and ORF2). We generated constructs including, under a CMV promoter, the ASAH2L transcript merged to GFP at the C-terminus in two frames corresponding to each of the two ORFs. We transfected HEK 293 cells and showed that both ORFs are recognized by the translation machinery and produce GFP tagged proteins, and thus it is possible that they are also translated in the brain. The GFP tagged ORF1 protein was found to be localized in the mitochondria, suggesting a possible role in oxidative phosphorylation or apoptosis, while the GFP tagged ORF2 protein was localized in the cytoplasm. Further work is necessary to show that translation indeed occurs in the brain for ORF1 and/or ORF2 and to determine the nature of their putative neuroprotective functions.

Investigating the neuropsychological phenotype of adult FMR1 premutation allele carriers. *J. Hunter[1], A. Abramowitz[2], M. Rusin[3], M. Leslie[1], G. Novak[1], D. Hamilton[1], L. Schubeck[1], K. Charen[1], S. Sherman[1]* 1) Department of Human Genetics, Emory University School of Medicine, Atlanta, Georgia; 2) Department of Psychology, Emory University, Atlanta, Georgia; 3) Independent practice, Atlanta, Georgia.

Expansion of the CGG repeat in the 5 untranslated region of FMR1, the fragile X mental retardation gene, is responsible for a spectrum of disorders. Repeats greater than 200, termed full mutation alleles, are associated with the mental retardation syndrome, fragile X. Expanded repeats in the range of ~55-199, termed premutation alleles, are associated with a late-onset tremor-ataxia syndrome most commonly in carrier males and primary ovarian insufficiency in carrier females. The neuropsychological impact of carrying a premutation allele is presently unclear in younger adults. In this study, we analyzed neuropsychological scores for 138 males and 506 females ascertained from the general population and from families with a history of fragile X syndrome. Subjects were age 18-50 years and had varying repeat lengths. Neuropsychological scores were obtained from measures of general intelligence, memory, executive functioning, and attention. Principal component analysis followed by varimax rotation was used to create independent factors for analysis: general intelligence, verbal ability, visual processing, self-report attention, sustained attention, and perseveration. These factors were modeled for males and females separately using a general linear model that accounted for correlation among related subjects. All models were adjusted for potential confounders, including age at testing, self-report ethnicity, and household income level. Among males, no repeat length associations were detected for any factor. Among females, significant associations with repeat length and general intelligence ($p=0.01$) and self-report attention ($p=0.03$) were detected, with premutation carriers having significant deficits compared to non-carriers. No significant interactions between repeat length and age were detected which would indicate that these deficits increase with age.

Down-regulation of gene expression at the Prader-Willi/Angelman syndrome imprinted domain by a paternally transmitted transgene. *E. Y. Smith, C. R. Futtner, A. J. DuBose, S. J. Chamberlain, J. L. Resnick* Molecular Genetics, University of Florida, Gainesville, FL.

Prader-Willi syndrome (PWS) and Angelman syndrome (AS) are distinct neurological disorders resulting from improper gene expression from the imprinted domain on chromosome 15q11-q13, the PWS/AS locus. This locus is controlled by a bipartite imprinting center consisting of the PWS-IC and the AS-IC. The PWS-IC is proposed to be a positive acting element that promotes gene expression from the paternal allele. The AS-IC acts on the maternal allele to inactivate the PWS-IC, thus silencing the paternally expressed genes. The PWS-IC is located just 5 to and including exon 1 of *SNRPN* whereas the AS-IC is 35 kb upstream of *SNRPN*. Importantly, the AS-IC includes one of several alternative upstream exons of *SNRPN*.

The PWS/AS locus is well conserved in the mouse, although the murine AS-IC remains poorly characterized. As in humans, the mouse *Snrpn* locus includes several upstream exons. We have taken a transgenic approach to study the potential regulatory role of these alternative exons. To do so, we utilized a bacterial artificial chromosome (BAC) containing *Snrpn* with approximately 100 kb of 5sequence encompassing three alternative exons. In low copy number, this transgene expresses *Snrpn* only upon paternal inheritance, suggesting that the BAC contains a functional AS-IC capable of inactivating the PWS-IC upon maternal inheritance. Targeted deletions of all three upstream exons lead to biallelic expression, suggesting inactivation of the AS-IC. When the two most 5 exons are deleted, imprinting is again lost. Surprisingly, paternal transmission of this transgene results in silencing of the endogenous *Snrpn* gene. Preliminary data show other paternally expressed genes are down-regulated as well. We are currently analyzing the remainder of the PWS/AS domain to determine whether the entire locus is repressed in addition to determining if aberrant epigenetic imprints are present at the endogenous locus. This model presents an exciting new approach to study imprinting at the PWS/AS locus.

Prenatal phenotypic variation in Ellis-van Creveld syndrome. *R. Lebel[1,2], L. Seaver[1], A. Gregg[3], J. Edwards[3], M. Salley[4], J. Avery[1], P. Broome[1], R. Stevenson[1]* 1) Greenwood Genetic Ctr, Greenwood, SC; 2) SUNY Upstate Medical University, Syracuse, NY; 3) University of South Carolina School of Medicine, Columbia, SC; 4) South Carolina OB-GYN Associates, PA, Columbia, SC.

A 21 year old womans first pregnancy ended with a blighted ovum (age 19); she presented with skeletal dysplasia (short tubular bones and ribs) and cardiac anomaly by ultrasound at 21 weeks; amnio was normal (46,XX). Autopsy confirmed prenatal report, adding endocardial cushion defect and polydactyly, supporting conclusion of Ellis-van Creveld syndrome (EC). Nine years later, after delivery of 2 normal children, she presented at 18 weeks with ultrasound findings of polydactyly, short tubular bones and ribs. Delivery was induced. Cells from the male fetus did not grow in culture. Autopsy was not consistent with EC because the cardiovascular defect was minor and there was hydrocephaly but no nuchal cyst, pulmonary hypoplasia or frenulum. No alternative diagnosis was clear but Majewski syndrome (MS) and dyssegmental dysplasia (DD) were considered. Seven months later she presented with cystic hygroma at 12 weeks. CVS showed 46,XX, but 2 weeks later intrauterine demise was found and delivery induced. Autopsy further expanded the anomaly list; syndrome assignment was reconsidered. The Beemer-Langer syndrome (BL) and Mucolipidosis type II (ML) were considered, but it was concluded that all 3 fetuses were affected by EVC, representing a broad phenotypic spectrum and illustrating the difficulties of prenatal syndrome recognition.

The Phenotypic Spectrum of Trisomy 2: A Case with Full and A Case with Mosaic Trisomy 2. *E. Mihci, G. Velagaleti, R. Ensenauer, D. Babovic-Vuksanovic* Mayo Clinic College of Medicine, Department of Medical Genetics, Rochester, MN.

Complete trisomy 2 is extremely rare and most reported cases were found during the prenatal period. The outcome of such pregnancies is highly variable, but spontaneous abortions are common with trisomy 2 accounting up to 1.1% of all spontaneous abortions. Only the mosaic form of trisomy 2 is observed in live-born individuals. In such cases, the postnatal period is characterized by growth delay, multiple congenital anomalies (including microcephaly, cleft lip and palate, facial dysmorphism) and developmental delay. We present two cases of trisomy 2. The first case, a 2-1/2-year old girl, presented with history of diaphragmatic hernia, duodenal atresia, intestinal malrotation, microcephaly, atrial and ventricular septal defect, developmental delay, hypotonia and seizures. Peripheral blood chromosome analysis showed normal 46,XX karyotype. Her phenotype was somewhat reminiscent of Pallister-Killian syndrome and skin biopsy was done. Chromosomal analysis on tissue fibroblasts showed mosaic trisomy 2 (3 out of 100 evaluated cells). Second case was an acardiac fetus in a monozygotic twin pregnancy. The normal twin was a baby girl with a karyotype 46,XX. Chromosome analysis of the Acardiac fetus showed complete trisomy with the karyotype 47, XX,+2. In conclusion, trisomy 2 is a rare chromosomal anomaly which can present with a varied phenotypic spectrum, the severity of which could be dependent on level of chromosomal mosaicism. Chromosome analysis of acardiac fetuses may help understand the etiology in some cases. Since trisomy rescue is a known phenomenon, in cases of rare mosaic trisomy 2, the phenomenon of trisomy rescue should be considered and uniparental disomy testing might help explain the phenotypic features, particularly in cases of discordant monozygotic twins.

The influence of continental axes of orientation on patterns of human gene flow. *S. Ramachandran*[1,2], *N. A. Rosenberg*[3,4,5] 1) Organismic and Evol. Biology, Harvard University, Cambridge, MA; 2) Society of Fellows, Harvard University, Cambridge, MA; 3) Department of Human Genetics, University of Michigan, Ann Arbor, MI; 4) Center for Computational Medicine and Biology, University of Michigan, Ann Arbor, MI; 5) The Life Sciences Institute, University of Michigan, Ann Arbor, MI.

Jared Diamond (Guns, Germs, and Steel, 1998) argued that the east-west orientation of the Eurasian landmass facilitated the rapid spread of agriculture and other technological innovations across Eurasia, while the north-south orientation of the Americas led to a slower diffusion of technology in the New World. We test Diamonds hypothesis with 678 microsatellite loci typed in 68 human populations from Eurasia and the Americas. If the diffusion of technology is accompanied by gene flow, then we expect genetic differentiation in the Americas along lines of longitude to be greater than that in Eurasia along latitudinal lines. We find that genetic differentiation is greater in the Americas than in Eurasia. Longitudinal distance explains a sizeable portion of genetic distance in Eurasia, while latitudinal distance does not explain a significant proportion of Eurasian genetic variation. Genetic differentiation in the Americas occurs along both latitudinal and longitudinal axes, suggesting the possibility of a greater degree of geographic complexity of gene flow in the Americas. These results support the view that migration patterns influenced by continental orientation have played an important role in determining the structure of human genetic variation and, by extension, the distribution and spread of cultural traits.

Mapping of a novel Ménières disease locus to chromosome 1q32.1-q32.3. *C. A. Campbell[1,2], J. Webster[3], C. Li[4], D. A. Stephan[3], R. J. H. Smith[1,2]* 1) Otolaryngology, University of Iowa, Iowa City, IA; 2) Interdepartmental PhD Program in Genetics, University of Iowa, Iowa City, IA; 3) Neurogenomics Division, Translational Genomics Research Institute, Phoenix, AZ; 4) Dept of Biostatistics, Harvard School of Public Health, Boston, MA.

Ménières disease (MD) is a complex disorder of unknown etiology characterized by vertigo, sensorineural hearing loss and tinnitus. Its reported incidence in Caucasians is 1-2 per 10,000 (Morrison 1995), but most cases of MD are sporadic and only occasional families are identified with multiple affected persons (Oliveira 1992). The dearth of such families and the reduced penetrance of MD make classic linkage analysis difficult.

We identified a Chilean family segregating autosomal dominant MD over three generations and completed a genome-wide linkage scan using the Affymetrix GeneChip Mapping 50K array. Five family members had definite MD, one individual had possible MD, 5 individuals were unaffected, and 4 individuals were too young to classify. Multi-point parametric linkage analysis assuming dominant inheritance and using DNA-Chip Analyzer software (dChip) (www.dchip.org) identified probable linkage to chromosome 1q32.1-q32.3 with a maximum LOD score of 2.36. The candidate gene interval determined by haplotype reconstruction spans 8.3 Mb (201.71- 210.29cM) and includes 79 known genes. No deafness locus maps to this region. The next linkage peak greater than 1 in this family was a LOD score of 1.66 at 17p12. This region spans 382 kb (12.65 - 13.03cM) and includes 6 genes.

Our study is significant because it identifies a locus for MD on 1q32. Since the phenotypes associated with sporadic and familial MD are indistinguishable, the identification of the causative gene in this family may have relevance to the definitive diagnosis of MD in sporadic causes. Furthermore, since little is known about MD and its initiating factors, the identification of a genetic contribution to this disease may help to clarify disease pathogenesis and possibly lead to better therapies.

Differentiation of renal oncocytoma and chromophobe renal cell carcinoma by interrogation of CCND1 by FISH and IHC: evidence that one-third of renal oncocytomas harbor a CCND1 abnormality. *A. W. Carlson, W. R. Sukov, R. P. Ketterling* Dept. of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN.

Renal oncocytoma (RO) is a kidney neoplasm with histologic features which can at times overlap with those of chromophobe renal cell carcinoma (ChRCC). As ChRCC is malignant and can be clinically aggressive, differentiating these tumors is important. Recent studies have shown cyclin D1 overexpression by immunohistochemistry (IHC) in a subgroup of renal oncocytomas. Fluorescence in situ hybridization (FISH) studies have also shown that oncocytomas may demonstrate rearrangement of the CCND1 locus. However, the frequency of cyclin D1 overexpression and CCND1 rearrangements and the clinical implications of these findings in RO or ChRCC has not been studied. We evaluated cyclin D1 overexpression by IHC and rearrangement of the CCND1 locus by FISH in formalin-fixed paraffin-embedded tissue sections from 63 RO, 36 ChRCC, and 25 non-neoplastic kidneys. Results of IHC and FISH for all 124 specimens were blinded until study completion. All 36 ChRCC and 25 non-neoplastic kidney specimens failed to show cyclin D1 overexpression or rearrangement of the CCND1 locus. Of the 63 RO specimens, 22 (35%) were abnormal for CCND1 by either IHC or FISH. Thirteen RO were abnormal for CCND1 both by FISH and IHC, 8 RO were abnormal for IHC only, and 1 RO was abnormal by FISH only. These results indicate Cyclin D1 overexpression and rearrangement of the CCND1 locus appears to be present in approximately one third of RO and absent in ChRCC, suggesting these tests may be helpful in distinguishing these two clinically disparate tumor types. In addition, these results suggest that the evaluation of CCND1 by both IHC and FISH may be warranted, since several cases had disparate results between these two methodologies. Further, since 8 RO demonstrated CCND1 overexpression by IHC but lacked a CCND1 rearrangement by FISH, the data indicates a genetic mutation, other than translocations or inversions, is responsible for the CCND1 overexpression in a subset of RO.

Sex Hormone Abnormalities in patients with Fibromuscular Dysplasia with Connective Tissue Features. *B. F. Griswold, L. Sloper, S. Basaria, J. M. Egan, N. B. McDonnell* National Institute on Aging Baltimore, Maryland.

We have identified a group of patients (n=28) who presented with arterial dissections and aneurysms as well as stenotic lesions with a diagnosis of fibromuscular dysplasia (FMD). Varying features of Ehlers-Danlos syndrome, such as atrophic scars, velvety or stretchy skin, joint hypermobility as evidenced by a high Beighton score, history of articular dislocations, uterine prolapse, joint pain, pectus deformities, pes planus and scoliosis were also present in this group of patients, and family history data suggested autosomal dominant inheritance with reduced penetrance. There was a female preponderance, 24 women and 4 men, consistent with prior observations that FMD affects women predominantly, leading to the hypothesis that sex hormones played a role in the penetrance of the disorder. As part of a longitudinal protocol studying the natural history of this disorder, laboratory analysis of sex hormone levels was completed. Of the 24 female patients aged 33-62, 15 were post-menopausal. Of the women subjects, 8/24 (0.33) had high levels of Sex Hormone Binding Globulin (SHBG) which included four post menopausal patients. Free testosterone was low in 36 percent; of women patients, and 4/24 (0.17) had low bioavailable levels of testosterone. Of the 8 patients found to have high levels of SHBG, five also had corresponding low free testosterone. None of the patients had liver function abnormalities or current birth control pill use which are known to increase SHBG. None of the premenopausal patients had low FSH or LH along with low estrogen which would suggest pituitary failure. By contrast, the four male patients had normal levels of testosterone and SHBG, however the sample size was too small to draw conclusions. Elevated SHBG and low testosterone in women with FMD may be a clue to pathways involved in the etiology of this disorder.

A comprehensive map of common copy number variation at 50bp resolution, and resulting biological insights. *D. F. Conrad[1], D. Pinto[4,5], L. Feuk[4,5], R. Redon[1], N. Carter[1], C. Lee[2,3], S. W. Scherer[4,5], M. E. Hurles[1]* 1) Sanger Institute, Cambridge, United Kingdom; 2) Brigham and Women's Hospital and Harvard Medical School, Cambridge, MA; 3) Broad Institute of Harvard and Massachusetts Institute of Technology, Cambridge, MA; 4) The Centre for Applied Genomics and Program in Genetics and Genomic Biology, The Hosptial for Sick Children, MaRS Centre, Toronto, Canada; 5) Department of Molecular and Medical Genetics, University of Toronto, Toronto, Canada.

Copy number variation (CNV) in the genome is extensive and yet is grossly under-ascertained, as there are only 1500 sites of common CNV presently known. We know that smaller CNVs are far more numerous than larger CNVs, and so improved CNV detection resolution can be expected to dramatically increase the numbers of known CNVs. The Genome Structural Variation Consortium has performed comparative genome hybridisation on a genome-wide set of tiling oligonucleotide arrays to discover the majority of common copy number variants >500bp in size in two populations with African and European ancestry. In addition we have generated data on a single chimpanzee to provide information on the ancestral state of observed variants. This array set covers the assayable portion of the human genome with 42,000,000 probes with a median spacing of ~50bp. These data reveal, as expected, that previous surveys captured only 5-10 percent of the CNVs within a single genome. The unparalleled resolution of these arrays precisely define the boundaries of over 10,0000 CNVs and so allow us to identify accurately functional sequences that are copy number variable, as well as providing new insights into the mechanisms generating chromosomal rearrangements. Current analyses suggest that our results represent a threefold increase in the number of known sites of common copy number variation. A custom CNV genotyping array has been designed off these discovery results and screening of the HapMap samples is underway.

Interaction between the NOS2A rs1060826 variant and smoking in neovascular age-related macular degeneration. *J. A. Ayala-Haedo[1], P. J. Gallins[1], P. Whitehead[1], K. L. Spencer[2], A. Argawal[2], E. Postel[3], J. L. Haines[2], M. A. Pericak-Vance[1], W. K. Scott[1]* 1) Miami Institute for Human Geno, University of Miami Miller School of Medicine, MIAMI, FL; 2) Center for Human Genetics Research, Vanderbilt University, Nashville, TN; 3) Duke University, Durham NC.

It has been previously demonstrated that SNPs in the NOS2A gene modulate the effect of smoking in neurodegenerative diseases. In particular, the rs1060826 synonymous SNP attenuates the protective effect of smoking in Parkinson disease (PD). Cigarette smoking is also a strong risk factor for age-related macular degeneration (AMD), with a demonstrated synergistic interaction with genotypes of the ARMS2 locus. The NOS2A gene is an attractive AMD candidate gene given its effect on smoking and its role in host defense and inflammation. The purpose of our study was to assess the possible main and interaction effects between rs1060826 genotype, AMD, and smoking. Using a Taqman assay, we genotyped rs1060826 in 260 cases of neovascular AMD (grade 5) and 145 unrelated controls with no macular changes (grade 1). Data were analyzed using a logistic regression model with rs1060826 genotype (dominant and recessive coding), age, sex, and smoking status (ever/never), along with a two-way interaction between rs1060826 and smoking. A significant interaction was detected between rs1060826 and smoking (p=0.03), and therefore we stratified the data set by rs1060826 genotype to examine modification of the effect of smoking by genotype. These stratified analyses demonstrated that the effect of smoking in AMD is stronger in carriers of the rs1060826 GG genotype (OR 4.1, 95% CI [1.9, 8.6]) than in those with the AG or AA genotypes (OR 2.1, 95% CI [0.94, 4.9]). The elevated risk of AMD in smokers with the GG genotype is similar to the effect seen in PD, where the GG genotype eliminates the protective effect of smoking. Our results support the hypothesis that SNPs in the NOS2A gene might modulate the effects of known disease risk factors. Our preliminary data suggests a more thorough analysis of the common variants in this candidate gene and their possible interactions with smoking and ARMS2 is necessary.

Integrative epigenetic regulation via MeCP2 mediated control over microRNA 137 in a mouse model of Rett Syndrome. *K. Szulwach[1], X. Li[2], R. Smrt[2], L. Li[1], Y. Li[1], Y. Luo[2], N. Santistevan[2], W. Li[1], X. Zhao[2], P. Jin[1]* 1) Dept Human Genetics, Emory University, Atlanta, GA; 2) Dept of Neuroscience, University of New Mexico, Albuquerque, NM.

Rett Syndrome (RTT) is an X-linked dominant progressive neurodevelopmental disorder caused by mutations in the gene encoding the DNA methyl-CpG-binding protein and transcriptional regulator, MeCP2. Identification of transcripts subjected to epigenetic regulation by MeCP2, particularly in the context of postnatal neurodevelopment, is critical toward understanding the molecular pathogenesis of RTT. microRNAs (miRNAs) function through the RNA interference (RNAi) pathway to post-transcriptionally regulate protein coding transcripts. Therefore, MeCP2 mediated epigenetic control over the expression of miRNA offers a means by which MeCP2 may contribute to the altered expression of protein coding genes, and thereby contribute to the molecular pathogenesis of RTT, without influencing mRNA transcription directly. We found that, within the postnatal neurodevelopmental context of adult neural stem cells (aNSCs), expression of specific miRNAs could be epigenetically regulated by MeCP2. We have demonstrated that one miRNA subjected to MeCP2 mediated epigenetic regulation, miR-137, modulates the proliferation and differentiation of aNSCs both in vitro and in vivo. Overexpression of miR-137 was found to promote the proliferation of aNSCs, whereas a reduction of miR-137 enhanced aNSC differentiation. Furthermore, miR-137 post-transcriptionally repressed the expression of Ezh2, a histone methyltransferase and a member of the Polycomb group (PcG) protein family important toward both stem cell function and neural development. Coexpression of Ezh2 rescued the phenotypes associated with miR-137 overexpression. These results demonstrate the integration of epigenetic regulation in adult neural stem cells via MeCP2 mediated control over miR-137 expression and downstream targeting of the epigenetic modulator Ezh2 by miR-137. Additionally, our data suggests that the loss of functional MeCP2 results in the altered expression of specific miRNAs, possibly contributing to the molecular pathogenesis of RTT.

Molecular features of karyotypically normal acute myeloid leukemia (AML). *A. Block[1], S. N. J. Sait[1], S. Kakati[1], P. Starostik[2], P. K. Wallace[3], M. Barcos[4], J. Marinaro[5], M. Wetzler[5], E. S. Wang[5], G. Deeb[4]* 1) Clinical Cytogenetics Lab, Roswell Park Cancer Inst, Buffalo, NY; 2) Molecular Diagnostics Lab, Roswell Park Cancer Inst, Buffalo, NY; 3) Laboratory of Flow Cytometry, Roswell Park Cancer Inst, Buffalo, NY; 4) Department of Pathology, Roswell Park Cancer Inst, Buffalo, NY; 5) Department of Medicine, Roswell Park Cancer Inst, Buffalo, NY.

Acute myeloid leukemia (AML) patients (pts) with a normal karyotype at diagnosis have been classified into an intermediate risk group, however, this subset of pts is heterogeneous in clinical outcomes. Since the accumulation of acquired genetic alterations and epigenetic changes in hematopoietic progenitor cells have been implicated in the development of AML, multiple submicroscopic genetic alterations with prognostic significance have been identified. In order to characterize molecular features of karyotypically normal AML, we combined analysis of fms-related tyrosine kinase 3 (FLT3) and nucleophosmin, member 1 (NPM1) mutation status in a group of 43 karyotypically normal AML pts treated at our institution. A 4 base pair insertion in NPM1 resulting in a reading frame shift was observed in 18/43 pts (9 female, 9 male; median age 71 yrs, range 34-88 yrs). 6/18 pts also had FLT3 internal tandem duplication (FLT3-ITD) and 2/18 pts had missense mutation of the aspartic acid of the FLT3 kinase domain (D835). FAB morphological classification included M1, M2, M4, M5a/b. One patient had a preceding myelodysplastic syndrome. Median WBC was 61.9 X 10^9/L (range 2.6-454.3). Based on available multiparameter flow cytometric analysis, $NPM1^+$ blasts lacked $CD34^+$ expression in 11/17 patients. FLT3 abnormalities alone were observed in 6/43 pts (1 female, 5 male; median age 56.5 yrs, range 34-81 yrs). Median WBC was 40.1 X 10^9/L (range 3.7-77.7) for these pts. Pts with NPM1 mutations in this study were older, presented with higher WBC, low/absent $CD34^+$ expression, FLT3 mutations, and without AML subtype specificity. Further identification of prognostic factors in karyotypically normal pts may optimize treatment and more precisely predict outcome.

Identification of aberrantly methylated genes in colon cancer that deregulate tumorigenic signaling pathways using methylated CpG island amplification and microarrays (MCAM). *K. D. Tsuchiya[1,2], S. Dzieciatkowski[1], J.-H. Lee[1], W. M. Grady[1,3]* 1) Clinical Research Div, Fred Hutchinson Cancer Research Ctr, Seattle, WA; 2) Dept of Labs, Children's Hosp and Reg Med Ctr, Seattle, WA; 3) Div of Gastroenterology, Univ of Washington School of Med, Seattle, WA.

Colorectal cancer (CRC) arises from the accumulation of mutations and epigenetic alterations in colon epithelial cells. It is well established that gene mutations promote CRC formation by deregulating signaling pathways. The tumor promoting consequences of mutations in genes such as APC have been demonstrated in cancer cell lines and in mouse models of intestinal cancer; however, the biological effects of most epigenetic events identified in CRC remain unknown. In addition, although many genes that are silenced by epigenetic alterations in CRC have been identified, evidence suggests that a large number remain to be discovered. The purpose of this study was to identify additional genes that are silenced by promoter methylation in CRC, and to assess whether genes regulating signaling pathways are commonly subject to aberrant DNA methylation in CRC. Genome-wide assays for aberrant DNA methylation were carried out on colon adenoma and adenocarcinoma cell lines by using Methylated CpG island Amplification followed by hybridization to microarrays containing probe coverage of promoter regions (MCAM). Methylated gene promoters identified by MCAM were validated by bisulfite sequencing of cell lines and primary colon tumors. MCAM identified a number of additional genes that are aberrantly methylated in colon neoplasm cell lines. A high percentage of these genes were confirmed to be heavily methylated in primary colon tumors. These genes included members of a variety of signaling pathways, including adrenergic receptor mediated signaling, PKC signaling, PI3K-AKT signaling, and others. The methylation of the majority of these genes is predicted to alter signaling in a pro-tumorigenic manner. Our results demonstrate that aberrant DNA methylation affects genes involved in a variety of signaling pathways in CRC, and that epigenetic alterations are likely a common mechanism for deregulating these pathways in CRCs.

Endophenotype-based approach identifies missense variant in *Transferrin* that influences A levels and risk for Alzheimer's disease. *A. Goate, J. S. K. Kauwe, J. Wang, K. Mayo, J. C. Morris, A. M. Fagan, D. M. Holtzman* Depts Psychiatry & Neurology, Washington Univ Sch Medicine, St Louis, MO.

Late-onset Alzheimers disease (LOAD) is the most common neurodegenerative disorder, affecting more than 5 million people in the United States alone. With the exception of apolipoprotein E epsilon 4 (*APOE 4*) no polymorphism has shown consistent and replicated association with LOAD. The creators of AlzGene, a publicly available online database, have used meta-analyses of published genetic association studies to address the problem of small sample sizes. In this study we have tested whether putative LOAD associated single nucleotide polymorphisms (SNPs) from the AlzGene meta-analysis are also associated with a novel quantitative endophenotype, cerebrospinal fluid (CSF) levels of 42 amino acid amyloid- (A42). Biomarker studies have shown that CSF A42 levels are correlated with LOAD status and A deposition, while genetic studies have shown that mutations causing early onset AD alter A42 levels. We genotyped 29 SNPs from 26 genes (from the AlzGene database on 12/01/07) in 313 individuals for whom CSF was collected after fasting. Genotypes were tested for association with normalized CSF A42/A40 ratio and CSF total A (CSF A40 plus A42) using analysis of covariance after adjusting for significant covariates. Alleles in *ACE, APOE, BDNF, DAPK1* and *TF* are associated with CSF A levels in a manner consistent with the previous association with LOAD. This suggests that these genes may influence risk for LOAD via an A dependent mechanism. Because the *TF* SNP (rs1049296) causes an amino acid substitution (P589S) we introduced the mutation into a cDNA and tested whether the SNP altered A42 levels *in vitro*. The minor allele of this polymorphism is associated with increased risk for LOAD (OR=1.18, CI:1.01-1.37), higher A42/A40 ratios in CSF (p=0.029) and higher A42/A40 ratios *in vitro* (p=0.0035). We conclude from this work that TF is a novel LOAD gene that influences risk via an A dependent mechanism. This work demonstrates the utility of this endophenotype-based approach in identifying SNPs that influence risk for LOAD.

BRD2, a JME susceptibility gene, is required for embryonic development in mouse. *E. Shang[1], X. Wang[3], D. J. Wolgemuth[2,3,4,5], D. A. Greenberg[1,6]* 1) Department of Biostatistics, Columbia University, New York, NY; 2) Department of Genetics and Development, Columbia University, New York, NY; 3) Department of Obstetrics and Gynecology, Columbia University, New York, NY; 4) The Herbert Irving Comprehensive Cancer Center, Columbia University, New York, NY; 5) The Institute of Human Nutrition, Columbia University, New York, NY; 6) New York State Psychiatric Institute, Columbia University, New York, NY.

Juvenile Myoclonic Epilepsy (JME) is one of the most common types of epilepsy, accounting for about 10% of all epilepsies. Linkage analysis and association studies have identified a susceptibility locus for JME as BRD2 on chromosome 6p21. We have generated a mutant mouse line lacking Brd2 function and showed that Brd2 is essential for embryonic development in mouse, especially for the development of the nervous system. Lacking the Brd2 gene causes embryonic lethality: most homozygous Brd2-/- embryos die by embryonic day 11.5. Prior to death, the homozygous embryos were notably smaller but the severest obvious abnormalities are seen in the developing brain where the gene is highly expressed. The neural structures of Brd2-/- mice are highly stunted and deformed, in contrast to other organs, which appear structurally intact. The heterozygous mice appear normal but are more sensitive to drug-induced seizure. We also observed spontaneous seizures in heterozygous individuals. The Brd2 knockout unambiguously demonstrates that the gene has a critical role in neurodevelopment.

Neurocognitive profile in older children with 22q11.2 Deletion Syndrome. *E. Chow[1,2], A. Ho[1], R. Higgins[3], S. Langlois[3], D. Young[1], A. Bassett[1,2]* 1) Centre for Addiction and Mental Health, Toronto, ON, Canada; 2) Dept Psychiatry, Univ Toronto, Toronto, ON, Canada; 3) BC Children's Hospital, Vancouver, BC, Canada.

22q11.2 deletion syndrome (22q11DS) is a genetic syndrome associated with deletions in chromosome 22. Approximately 25% of adults with 22q11DS develop schizophrenia (SZ) (Bassett et al, 2005). A comprehensive study on the neurocognitive profile of 22q11DS adults reported no difference in IQ but greater impairments in motor function, learning and memory, verbal fluency and social cognition in subjects with SZ than those without SZ (Chow et al, 2006). In this study, we compared performance between children with 22q11DS and their non-deleted siblings in cognitive domains similar to this study. Thirty children with 22q11DS (13 M 17 F; age M = 10.0 years, SD = 2.0 years; IQ M = 68.0, SD = 13.7), and 12 non-deleted siblings (6 M, 6 F; age M = 10.9 years, SD = 1.5 years; IQ M = 96.0, SD = 20.0) were included in the study. All subjects were assessed with the Connors Continuous Performance Test (CPT), the Picture Arrangement subtest of the Wechsler Intelligence Scale for Children III (WISC III), the Rey-Auditory Verbal Learning Test (RAVLT), Animals naming, the Purdue Pegboard Test, Theory of Mind (TOM), and Judgement of Line Orientation (JOL). Performance on the attention index of the CPT, Picture Arrangement subtest, 5-trial total recall of the RAVLT, bilateral dexterity in the Pegboard test, Animals naming, TOM, and JOL were compared between the subject groups. The two groups differed significantly on multivariate analysis ($F(7, 26) = 16.75$, $p = <0.001$), and post-hoc Tukey-Kramer's corrections for multiple comparisons yielded significant effects for 5 tests: Picture Arrangement ($p = <0.001$), RAVLT total recall ($p = 0.021$), Animal naming ($p = 0.002$), bilateral dexterity score ($p = <0.001$), and JOL ($p = 0.001$). Interestingly, 5 22q11DS subjects (17%) performed poorly in at least 4 of the 7 test variables. These individuals may potentially be at the most risk for developing SZ in adulthood; follow-up studies with this sample will be needed to assess whether this relationship will come true.

Comparative analysis of whole-genome genotyping arrays and candidate gene arrays for copy number variation detection. *K. Wang[1,3], B. Keating[2], J. Glessner[3], T. Cappola[4], M. Bucan[1], H. Hakonarson[3], D. Rader[4], M. Reilly[4], M. Li[5]* 1) Dept Genetics, Univ Pennsylvania, Philadelphia, PA; 2) Dept of Pharmacology, Univ Pennsylvania, Philadelphia, PA; 3) Center for Applied Genomics, Children's Hospital of Philadelphia, Philadelphia, PA; 4) Dept of Medicine, University of Pennsylvania, Philadelphia, PA; 5) Dept of Biostatistics and Epidemiology, Univ of Pennsylvania, Philadelphia, PA.

Genome-wide and candidate gene association studies have been routinely used for gene mapping. In addition to SNP-based analysis, the analysis of copy number variations (CNVs) may provide novel and complementary insights into the genetic susceptibility of complex diseases. In this study, we aim to test the relative merits of using genome-wide approach and candidate gene approach for the detection of CNVs. We have assembled a coronary artery disease (CAD) case-control study of 421 acute CAD cases, 454 chronic CAD cases and 447 angiographically normal controls. We genotyped this cohort using the Affymetrix genome-wide 6.0 array with ~900K SNPs and ~900K non-polymorphic markers, as well as the Illumina ITMAT-Broad-CARE (IBC) array with ~50K densely spaced SNPs in ~2,000 candidate genes, including 500 priority 1 genes that are densely covered. Since the IBC array has much better coverage for these genes than the Affymetrix 6.0 array, our data provide a unique opportunity for validating CNVs detected in Affymetrix and for refining boundaries of small-sized CNVs. We adapted PennCNV, a hidden Markov model based algorithm, to both the Affymetrix and Illumina arrays. A total of 474 Affymetrix CNV calls can be covered by at least 10 SNPs in the IBC array. Of these, 225 Affymetrix CNV calls are larger than 100kb, and 119 (53%) can be validated by IBC; the rate of validation increases to 40/53 (76%) if we restrict the analysis to Affymetrix CNV calls that are larger than 500kb. Our results suggest that the combined analysis of two technical platforms can provide complementary information in validating CNVs. Results from our analysis can be directly used in subsequent association tests which may provide insights into the genomic architecture of human cardiovascular diseases.

Optimizing Bisulfite DNA Conversion Method for Methylated CpG Island Discovery and Screening. *E. Currie-Fraser, B. Finkelnburg, V. Boyd, M. Barker, M. Bozzini, S. Jankowski, L. Xu* Applied Biosystems, Foster City, CA.

DNA methylation at the 5 position of cytosine in CpG islands plays a critical role in the epigenetic regulation of gene expression. Typically methylation is inversely correlated with the transcription status of the gene. Bisulfite DNA conversion is one of the most used techniques for methylation studies because of its relative simplicity, whereas other methods are frequently cumbersome and require significant optimization. The bisulfite conversion method allows precise analysis of methylation in a target region by converting all nonmethylated cytosines into uracils, while methylated cytosines remain unchanged. The workflows described here provide an effective solution for methylation analysis with straightforward protocols. There are many different approaches to methylation analysis, and in this poster we describe 3 different options. We demonstrate that a candidate regions methylation state and genotype information for each CpG locus across the entire candidate region can be identified with confidence using an optimized approach, which requires small amounts of genomic DNA and generates high quality data. These workflows, therefore, are very useful for the analysis of samples where the amount of material is limited, and when analysis time is an important factor.

Cis-regulatory elements in the Epidermal Differentiation Complex (EDC): Towards understanding atopic dermatitis and psoriasis. *C. de Guzman Strong[1], K. Sears[2], J. Segre[1]* 1) National Human Genome Research Institute, Bethesda, MD; 2) University of Illinois at Urbana-Champaign, Urbana, IL.

The Epidermal Differentiation Complex (EDC) locus (1q21) harbors a set of genes that are specifically expressed upon epidermal differentiation and barrier formation. Atopic dermatitis (AD) and psoriasis are common skin inflammatory disorders that both share independent linkage to the EDC suggesting a role for these genes in disease etiology. Recently, mutations in filaggrin (FLG) in the EDC are strongly linked to familial ichthyosis vulgaris and are highly associated with cases of AD that often advance to asthma (atopic march). AD familial studies excluding FLG continue to demonstrate EDC linkage suggesting additional genetic variants within the EDC in AD pathogenesis. The proximity and density of genes in the EDC suggest coordinate regulation via cis-regulatory elements for temporal and spatial epidermal expression. We hypothesize that evolutionarily conserved noncoding sequences (CNS) function as cis-regulatory elements to coordinate transcriptional regulation of the EDC genes. A genomics approach using comparative multi-species sequence analysis (MultiPip) of the EDC loci from human, chimpanzee, rhesus, mouse, rat, dog, and opossum genomes has identified 43 CNS as potential regulatory elements. The use of the opossum genome as a stringent criterion in annotating CNS in the EDC has been confirmed based on the evolutionary conservation of dorsal to ventral patterning of epidermal barrier acquisition in the opossum. Our functional analysis of the CNS using dual luciferase reporter assays in differentiating keratinocytes has identified either enhancer or repressor activity in roughly 50% of the CNS. We are currently correlating our findings with Genetic Association Information Network (GAIN) psoriasis studies. Taken together, our results suggest a high proportion of regulatory activity in the CNS that may coordinate expression of the genes in the EDC and could pose as genetic variants contributing to either AD or psoriasis.

Disruptions of distant regulatory elements outside *FOXL2* in BPES syndrome: exploring the role of microdeletions and variants in Conserved Non-Coding sequences (CNCs). *E. De Baere[1], B. D'haene[1], C. Attanasio[2], D. Beysen[1], M. Friedli[2], B. Lorenz[3], P. Lapunzina[4], B. Lowry[5], T. de Ravel[6], W. Reardon[7], G. Pierquin[8], A. Trainer[9], R. Fisher[10], S. Del Pozo[11], W. Courtens[12], M. Field[13], P. Bouchard[14], B. Menten[1], A. De Paepe[1], S. E. Antonarakis[2]* 1) Ctr Med Genet, Ghent Univ Hosp, Belgium; 2) Dept of Genet Med and Dev, Univ of Geneva Med School, Switzerland; 3) Dept of Opthalmol, Univ Hosp Giessen, Germany; 4) S de Genet Med y Mol and CIBERER, ISCIII, Spain; 5) Dept of Med Genet, Alberta Children's Hosp, Canada; 6) Ctr Hum Genet, Leuven Univ Hosp, Belgium; 7) Nat Ctr Med Genetics, Our Lady's Hosp for Sick Children, Ireland; 8) Dept Hum Genet, Sart Tilman, Belgium; 9) Inst Hum Genet, Univ of Newcastle, UK; 10) St James's Univ Hosp, UK; 11) Dept of Ped, Hosp 12 de Oct, Spain; 12) Ctr Hum Genet, UCL, Belgium; 13) North Clin School, Univ of Sidney, Australia; 14) Dept of Endocrin, Hosp St-Antoine, France.

Blepharophimosis syndrome (BPES) is a development disorder caused by *FOXL2* mutations, deletions or long-range microdeletions. In 12% of patients however, the molecular defect remains unknown. The major aim of this study was to unravel the genetic defect in 40 patients without *FOXL2* mutations/deletions. For this we developed targeted arrays, quantitative PCR (qPCR) and sequencing of CNCs located in previously described 5' microdeletions. ArrayCGH and qPCR revealed 1 and 3 new microdeletions 5' of *FOXL2* respectively. High-resolution qPCR and sequencing of CNCs showed that subtle copy number changes or point mutations in CNCs do not play a major role in the causation of BPES. Second, we identified and delineated 11 new deletions using microsatellites, arrayCGH and qPCR, and further characterized 10 known deletions. Overall, deletions found in the *FOXL2* region are characterized by scattered breakpoints, suggesting NHEJ or FoSTeS as possible underlying mechanisms. In conclusion, our study emphasizes the importance of disrupted long-range transcriptional control in the molecular pathogenesis of BPES. Finally, it shows the need for exhaustive copy number screening of the *FOXL2* region in this development disorder.

Genomic regions influencing plasma levels of resistin in African-Americans and non-Hispanic whites from hypertensive sibships. *K. Ding[1], M. de Andrade[2], S. L. R. Kardia[3], S. T. Turner[4], T. H. Mosley,Jr[5], I. J. Kullo[1]* 1) Division of Cardiovascular Diseases, Mayo Clinic, Rochester MN; 2) Department of Biostatistics, Mayo Clinic, Rochester MN; 3) Department of Epidemiology, University of Michigan Ann Arbor, Ann Arbor MI; 4) Division of Nephrology and Hypertension, Mayo Clinic, Rochester MN; 5) Department of Medicine, University of Mississippi, Jackson MS.

Resistin, a proinflammatory cytokine produced by macrophages in adipose tissue, has been implicated in insulin resistance and plasma resistin levels have been associated with vascular disease phenotypes. We investigated genetic determinants of plasma resistin levels in 1480 African-Americans (AAs) and 1230 non-Hispanic whites (NHWs) belonging to sibships ascertained on the basis of hypertension. Plasma resistin levels were measured by solid-phase sandwich ELISA and adjusted for conventional risk factors, serum creatinine, alcohol use, and physical activity, prior to genetic analyses. Heritability of plasma resistin levels was calculated as the proportion of the total phenotypic variance due to additive genetic effects. Genome-wide linkage analyses were performed using ~360 autosomal microsatellite markers and a variance components approach. Association analyses were performed using 11 tag SNPs in the resistin gene (*RETN*), with adjustment for presence of sibships. Plasma levels of resistin were highly heritable: $h^2 = 0.61$ in AAs, and 0.58 in NHWs. Two adjacent linkage signals were noted on chr 19p13 in AAs (LOD = 5.28 and 5.64, respectively), one of which harbors *RETN*. Tentative evidence of linkage was present on chr 6 (LOD = 1.60) in NHWs. SNP rs1862513 in the promoter region ($P = 0.00001$) and SNP rs1477341 in the 3 flanking region ($P = 0.0001$) were associated with resistin levels in AAs. SNP rs1477341 was weakly associated with resistin levels in NHWs ($P = 0.03$). Conclusion. Plasma resistin levels are highly heritable. We identified two quantitative trait loci for plasma resistin levels on chr 19p13 in AAs, one of which harbors *RETN*, and two SNPs in *RETN* that were associated with resistin levels.

ROOTLESS SYNDROME : A CASE WITH A LARGE SPECTRUM OF DENTIN DYSPLASIA. *B. DEMEER[1], C. TOUGNE[2], G. MORIN[1], B. RICHARD[3], S. DE BROCA[2], B. ARVEILER[3], M. MATHIEU[1]* 1) Department of Genetics, University hospital, Amiens, France; 2) Department of Maxillofacial Surgery, University Hospital, Amiens, France; 3) Department of genetics, University Hospital, Bordeaux, France.

We report a 10 year old boy with generalized yellow discoloration of the teeth, unusual mobility and premature loss of teeth. Both primary and secondary dentitions were affected. The clinical shape of the crowns of the teeth appeared to be normal. On clinical examination neither ectodermal defect, nor skeletal or articular features were found. Panoramic radiograph features included rootless teeth, short and abnormal shaped root teeth. In this family, the probands grand-mother and father, one of his aunt and her two boys were also affected, expressing an autosomal dominant transmission. According to the clinical and radiological phenotype, the diagnosis of dentin dysplasia type I is likely. Hereditary dentin defects have historically been classified into two main groups : dentin dysplasia (DD) and dentinogenesis imperfecta (DGI). According to this classification system, based upon clinical and radiographic features, isolated inherited dentin defect are categorized as DGI types II and III, and DD types I and II. To date, mutations in DSPP-Dentin Sialophophoprotein- have been found to underlie the dentin disorders DGI types II and III and DD type II. Dentin dysplasia type I, also known as rootless teeth (OMIM 125400) or radicular dentin dysplasia, is a very rare autosomal dominant genetic disease of unknown aetiology, causing incomplete tooth formation that results in premature exfoliation of both the primary and permanent dentitions. Study of DMP1 gene -Dentin matrix acidic phosphoprotein- and DSPP gene, except a part of exon 5 of DSPP due to technical reasons, has been performed, and no mutation was found.

The putative type 2 diabetes association signals in the *NOSAP1* and *PKLR* gene regions on chromosome 1q are not confirmed by a large scale follow up study. *N. W. Rayner[1], I. Prokopenko[1], C. J. Groves[1], E. Zeggini[1], R. L. Hanson[2], B. D. Mitchell[3], W. Jia[5], M. Ng[6], P. Froguel[4], J. Chan[6], C. Bogardus[2], S. C. Elbein[7], A. R. Shuldiner[3], M. I. McCarthy[1] For the International Type 2 Diabetes Consortium* 1) Oxford, United Kingdom; 2) Phoenix, AZ, USA; 3) Baltimore, MD, USA; 4) Lille, France; 5) Shanghai, China; 6) Hong Kong, China; 7) Little Rock, AR, USA.

High density SNP mapping (mean density 4.3kb) of the well replicated chromosome 1q type 2 diabetes (T2D) linkage region (147.0-169.7Mb) identified two association signals in samples of European descent. The first resides in the *NOS1AP* (*CAPON*) gene region (e.g. rs7538490, OR 1.53 [95%CIs 1.28-1.81], $p=1.2x10^{-6}$) and the second within a region of extensive linkage disequilibrium (LD) that includes the *ASH1L* and *PKLR* genes (e.g. rs11264372, OR 1.35 [1.17-1.56], p $5.1x10^{-5}$).

We genotyped rs7538490 in four independent sets of European origin consisting of a total of 5509 T2D cases and 7843 controls, we also genotyped rs11264371 (r^2~1.0 with rs11264372) in the same four independent sets along with a fifth set giving a total of 6067 T2D cases and 9878 controls.

The replication in these UK case-control sets failed to demonstrate any detectable effect at either SNP. Meta-analysis of the combined replication sets showed no association with T2D at these two loci: rs7538490, OR 1.01 [0.96-1.07], p 0.63, rs11264371, OR 1.04 [0.99-1.11], p 0.14. We conclude that no convincing role in T2D susceptibility was observed for these SNPs in *NOSA1P* and the *ASH1L*/*PKLR* gene region, notwithstanding any differences in the ascertainment of the replication cases and the highly selected cases used for the original screen. All in all, this large, well powered, dense association study has failed to identify any common SNP variants that may be contributing to the replicated linkage signal and we are now investigating whether structural variants, and low frequency intermediate penetrance SNPs are responsible for the observed linkage signal.

New Haplotype Sharing Method for Genome-Wide Case-Control Association Studies Implicates Gene for Parkinsons Disease. *G. A. Satten[1], A. S. Allen[2]* 1) Centers for Disease Control and Prevention, Atlanta, GA; 2) Department of Biostatistics and Bioinformatics and Duke Clinical Research Institute, Duke University, Durham NC.

The large number of markers considered in a genome-wide association study (GWAS) has resulted in a simplification of analyses conducted. Most studies are analyzed one marker at a time using simple tests like the trend test. Methods that account for the special features of genetic association studies, yet remain computationally feasible for genome-wide analysis, are desirable as they may lead to increased power to detect associations. Haplotype sharing attempts to translate between population genetics and genetic epidemiology. Near a recent disease-causing mutation, case haplotypes should be more similar to each other than control haplotypes. We give computationally simple association tests based on haplotype sharing that can be easily applied to GWASs while allowing use of fast (but not likelihood-based) haplotyping algorithms and properly accounting for the uncertainty introduced by using inferred haplotypes. We also give haplotype sharing analyses that adjust for population stratification. Applying our methods to a GWAS of Parkinsons disease, we find a genome-wide significant signal in a biologically-plausible gene that is not found by single-snp methods. Further, a missing-data artifact that causes a spurious single-SNP association on chromosome 9 does not impact our test.

Mechanisms and clinical significance of copy number changes (CNCs) detected by aCGH in a pediatric patient population. *E. L. Baldwin[1], E. C. Thorland[2], A. R. Brothman[3], C. L. Martin[1], D. H. Ledbetter[1]* 1) Department of Human Genetics, Emory University School of Medicine, Atlanta, GA; 2) Cytogenetics, Department of Lab Medicine & Pathology, Mayo Clinic, Rochester, MN; 3) Departments of Pediatrics and Pathology, University of Utah Health Sciences Center, Salt Lake City, UT.

Significant data are accumulating on the frequency, size and location of submicroscopic copy number changes (CNCs) in normal and various disease-specific populations. However, there is still a lack of information on the mutation rate and mechanism of origin of these duplication and deletion events, as well as the factors contributing to their potential clinical significance. We have analyzed >1200 patients with developmental delay/mental retardation/autism by aCGH using a custom-designed oligonucleotide array with whole genome plus targeted coverage. This array design has been validated and clinically implemented in a multi-center consortium, and will accurately detect genomic imbalances >300 kb anywhere in the euchromatic genome. A subset of copy number gains (CNGs) and losses (CNLs) 300 kb-10 Mb in size with known inheritance was selected for analysis of mechanism and clinical significance. Of 65 imbalances, only 15% were mediated by flanking segmental duplications (seg dups), while 85% were not associated with seg dups and represent random chromosome breakage. Twenty-six imbalances were inherited from a normal parent and were considered benign CNCs, whereas 28 imbalances were *de novo* and considered likely pathogenic CNCs. For the benign CNCs, duplications were more frequent than deletions (61.5% vs. 38.5%). However, for pathogenic CNCs, deletions were significantly more frequent (82.1%). The average size of pathogenic CNCs was 2.5 Mb, while the average size of benign CNCs was 946 kb. For a subset of imbalances in the size range of 300 kb-3 Mb, we found an average of 2 genes for the benign CNCs compared to an average 9 genes for the pathogenic CNCs, consistent with the expectation that imbalances involving a larger number of genes are more likely to include dosage sensitive genes producing phenotypic effects.

Investigation of non-coding regulatory elements as targets of Type 2 Diabetes susceptibility regions. *P. Akan[1], C. Lindgren[2], I. Prokopenko[2], M. McCarthy[2], P. Deloukas[1]* 1) Wellcome Trust Sanger Institute, Genetics of Complex Traits in Humans, Hinxton Cambridge, CB10 1SA, UK; 2) Oxford Centre for Diabetes, Endocrinology and Metabolism (OCDEM) Churchill Hospital, Old Road Headington, Oxford, OX3 7LJ, UK.

Type 2 Diabetes (T2D) is a complex metabolic disease characterised by elevated serum glucose levels, mainly caused by defects in insulin action, secretion or both. The manifestation of the disease does not follow Mendelian segregation however; twin studies suggest strong heterogeneous genetic component acting collectively with environmental risk factors for disease predisposition (average concordance rates are 58.8% and 26.8% in monozygotic and dizygotic twins respectively). The genetic component of the disease is highly heterogeneous; several loci in the genome contributing moderate levels to the disease. Over 10 linkage scans of T2D in multiple populations have given a signal at chr1q21-25. As part of the International 1q consortium, we have undertaken a systematic investigation of regulatory elements in this region which spans 26 Mb. We are applying chromatin immunoprecipitation (ChIP) methodology using 13 antibodies against a combination of modified histones and transcription factors that mark promoters, enhancers and insulators. ChIP experiments are being performed in human primary liver epithelial (THLE-2), primary skeletal muscle and pancreatic beta cells (Hap-5). ChIPed material is analysed using a custom-made microarray with 50 bp resolution covering the entire region. To date, we have identified 591 (117 intra-genic) potential regulatory elements; only 198 of them were within 2 kb of a known transcription start site. Moreover, we have found 124 potential promoter elements in the region (at least 10 kb away from any known transcription start site), their functional investigation is ongoing. Our present goal is to sequence such potential regions in multiple individuals with different case control status to establish their functional importance in disease progress.

Congenital Hepatic Fibrosis: A common feature in various ciliopathies. *L. Lukose[1], T. Heller[3], M. Parisi[4], P. Choyke[5], K. Daryanani[6], B. Turkbey[7], J. Bryant[1], G. Golas[1], K. O'Brien[1], A. Garcia[1], D. Adams[1], L. Guay-Woodford[7], P. Mohan[8], W. A. Gahl[1,2], M. Gunay-Aygun[1,2]* 1) Medical Genetics Branch, NHGRI, NIH, Bethesda, MD; 2) Intramural Program of the Office of Rare Diseases, DHHS; 3) NIDDK, Bethesda, MD; 4) University of Washington, Seattle, WA; 5) Molecular Imaging Program, NCI, Bethesda, MD; 6) NIH Clinical Center, Bethesda, MD; 7) University of Alabama, Birmingham, Al; 8) CNMC, Washington, DC.

Congenital Hepatic Fibrosis (CHF) is a unique, genetically-determined liver pathology, caused by defective remodeling and branching of the developing biliary and portal system referred to as ductal plate malformation (DPM). Although CHF is commonly associated with autosomal recessive polycystic kidney disease (ARPKD), it can be part of many other ciliopathies including Joubert syndrome and related disorders (JSRD), Bardet-Biedl (BBS), Meckel-Gruber (MKS), oral-facial-digital (OFD) syndromes, and skeletal dysplasias caused by ciliary defects. Through our ongoing NIH trial on ciliopathies (ClinicalTrials.gov, number, NCT00068224), we evaluated 110 CHF patients (85 ARPKD/CHF, 5 ADPKD/CHF, 10 JSRD/CHF, 10 unknown type PKD/CHF) some followed prospectively for up to 5 years. In contrast to the findings in cirrhosis, liver synthetic function in CHF was intact and liver enzymes were largely normal. Portal hypertension (PH) resulting in splenomegaly and esophageal varices was the most common clinical problem. Decreased platelet count due to hypersplenism, correlated inversely with spleen volume, making this parameter a reliable indicator of the severity of PH. The severity of kidney and liver disease were independent of each other, since creatinine clearance did not correlate with spleen volume. A subset of JSRD patients had a variant form of CHF with elevated hepatocellular and biliary enzymes. Most CHF patients, especially those with ARPKD had intra- and extrahepatic gross biliary dilatations. Longer follow up and accurate molecular diagnostic classification of this cohort of CHF patients will allow us to better define the liver phenotype of ciliopathies.

Hunter Syndrome: Functional/Cognitive Improvement in Severe Cases with Enzyme Replacement Therapy. *S. Root* Peds, Univ New Mexico Sch Med, Albuquerque, NM.

This paper describes two patients with Hunter Syndrome, aged 9 and 13. Both had cognitive decline, progressively losing functional skills. By the time enzyme replacement therapy was FDA-approved, both had lost functionality, losing most speech and ambulation. By two months on treatment, both showed resolution of their hepatosplenomegaly, with improved mood and affect. Both regained some speech, along with improved ambulation and use of hands. Patient A has had frequent hospitalizations with respiratory illnesses and/or sepsis, and had a tracheotomy by age 3. He began weekly enzyme replacement with multiple episodes of presumed infusion reactions, with red skin and hives appearing, then subsiding, with or without treatment. He was started on montelukast, and these episodes mostly disappeared. His liver and spleen shrank to normal. He now blurts out single words, appropriate to the situation. He is able to walk long distances. At school, he participates in class effectively, and even says a few words that others can understand. He can now complete a task once his teachers start it off with brief hand-over-hand work. Patient B never needed hospitalization, but had herniorrhaphies. Good health, but showed evidence of developmental delay, and before enzyme replacement therapy was approved he had lost all speech and was unwilling to walk very far. He used his hands like spades, unable to functionally separate his fingers. On therapy, his hepatosplenomegaly quickly resolved and his tongue shrank. He learned to catch and throw a ball accurately. He rarely uses a word, but understands much of what is said to him. His favorite activity is watching Tom and Jerry cartoons, and he can follow the plot. Hes able to walk for miles. On montelukast, his chronic cough and rhinorrhea disappeared. Enzyme replacement therapy has transformed the lives of both these children. It is not a cure, but has given them more function and their families hope. What is responsible for the improvementsremoval of mucopolysaccharides in the brain? Loss of hepatosplenomegaly? Improved joint function? Smaller tongue size? Is some due to the anti-inflammatory effects of montelukast? They and their families are pleased.

Realizing single molecule clinical genetics. *M. Boyce-Jacino, P. Deshpande, N. Fernandes, L. Changolkar, MD. Austin, S. Vijayan, H. Cao* BioNanomatrix Inc., Philadelphia, PA.

Advances in understanding genetic complexity from SNPs to gene duplications and rearrangements to regulatory changes in methylation have driven the need for increasingly sensitive and accurate methods of DNA analysis to ensure informative association of DNA variation with clinical status. Ideally, such methods would enable direct analysis of high molecular weight genomic DNA in native-state constructs. To this end, we have been developing a simple and robust approach to sorting single DNA molecules in a massively parallel fashion, enabling direct imaging of structural barcodes of individual long (100,000-200,000 bp) genomic DNA fragments in a continuous flow mode. Our approach utilizes a nanochannel chip capable of parallel sorting and single molecule analysis of megabase DNA molecules. The chip consists of an array of enclosed nanochannels which enable linearization and prevents individual molecules from folding back on themselves while inside the channel. The chip provides a linearized DNA molecule that is suspended in solution in its native helical form. The samples tested to date include native genomic DNA, bacterial artificial chromosomes and long range PCR products. We present results showing that our nanochannel device can be used to perform sizing of genomic DNA fragmented by radiation damage or apoptosis, to determine the distances between fluorescently labeled sequence-specific biomarkers for genomic mapping/structural variation detection, and to determine long range haplotypes. The device can even examine protein binding events for epigenetic studies on molecules that are up to 200 kbp and larger, at sub-kilobase resolution. Capabilities of the system and mapping results will be described.

Sacral agenesis, T-box genes, and independent evolution of short tails in Manx Cats. *K. J. Buckingham[1], M. J. McMillin[1], M. L. Feldkamp[2], M. J. Bamshad[1]* 1) Dept. of Pediatrics, University of Washington, Seattle, WA, USA; 2) Dept. of Pediatrics, University of Utah, Salt Lake City, UT, USA.

Tails in vertebrates are used for a wide variety of functions including balance, locomotion, and communication. Both within and among species variation in tail length is common and phylogenetic analyses suggest that shortening or loss of the tail has occurred multiple times independently. The evolutionary mechanism(s) that explains this observation is, as of yet, unknown. Tail length variation is particularly common among different breeds of domestic cat. Notably the tail of the Manx cat can be categorized into four phenotypes that range from absence of the tail (i.e., rumpy), to a minimal tail (i.e., rumpy riser), to a short tail (i.e., stumpy), to a full tail. The variable tail length of the Manx recapitulates that of mice heterozygous for deletions of the canonical T-box gene, *T*, which encodes the transcription factor Brachyury, a key regulator of notochord differentiation in all vertebrates. We resequenced *T* in several independent lineages of Manx cats and identified multiple small deletions, each predicted to cause a frameshift that leads to premature termination and truncation of the carboxy terminal end of Brachyury. Mutant alleles appeared to be largely lineage specific, all of the animals with shortened tails were heterozygous for *T* mutations, and none of the animals with a *T* mutation had a tail of normal length. One of these alleles was shared with American Bobtails, another cat breed with a short tail. These observations indicate that variable Brachyury dosage might be a general mechanism for influencing tail length in vertebrates. Moreover, Manx cats with shortened tails have a higher incidence of imperforate anus and vertebral defects such as sacral agenesis. A missense mutation predicted to substitute a conservative amino acid in the carboxy terminal domain of Brachyury was recently reported in a case of sacral agenesis and screening of a modestly sized cohort of cases for additional mutations is underway. These results suggest that *T* variants might also influence the risk for birth defects in humans.

New Mutations in GDAP1 gene are responsible for axonal autosomal recessive Charcot-Marie-Tooth disease (ARCMT2)in families from Sudan and Yemen. *H. Azzedine[1,2], M. A. M. Salih[3], E. Mikesova[1], S. A. Elmalik[4], M. M. Kabiraj[5], A. E. M. Ahmed[6], M. M. Mukhtar[7], E. LeGuern[1,8]* 1) INSERM U679, Hôpital de la Pitié-Salpêtrière, Paris, France; 2) National Centre for Neurogenetic disorders, CHU Angers, Angers, France; 3) Division of Pediatric Neurology, Department Of Pediatrics, College of Medicine, King Saud University, Riyadh, Saudi Arabia; 4) Department of Physiology, College of Medicine, King Saud University, Riyadh, Saudi Arabia; 5) Division of Clinical Neurophysiology, Department of Neuroscience, Armed Forces Hospital, Riyadh, Saudi Arabia; 6) Department of Physiology, Faculty of Medicine, University of Khartoum, Sudan; 7) Institute of Endemic Diseases, Faculty of Medicine, University of Khartoum, Sudan; 8) UF de génétique, Département de Génétique et Cytogénétique, Hôpital de la Pitié-Salpêtrière, Paris, France.

CMT is a pathological and genetic heterogeneous group of hereditary motor and sensory neuropathies characterized by slowly progressive weakness and atrophy, primarily in peroneal and distal leg muscles. Two major types have been distinguished on pathological and electrophysiological grounds: demyelinating and axonal CMT. We describe 3 consanguinous Sudanese and Yemeni families with ARCMT2. These consisted of 16 individuals aged 2-35 years and there parents. Five of them were affected. Onset was about 2 years with delayed walking, evolving into high steppage gait and distal lower limb muscle weakness and wasting. Cognitive development was normal, ambulation was preserved and no scoliosis was detected. Nerve conduction studies were compatible with axonal CMT. However, nerve pathology showed prominent loss of large myelinated axons and typical onion bulbs formations. These families were screened for 12 microsatellites markers covering the 2 more frequent ARCMT2 loci (CMT4A/2K and CMT2B1). Assignment of the families to the CMT4B/2K was established by homozygocity mapping. Sequencing of GDAP1 was performed in all members of the 3 families and 3 new mutations were identified. In our Knowledge, These are the first GDAP1 mutations reported in Sudanese and Yemeni families. The phenotype is of axonal type, compatible to the majority of previously reported studies.

Protective effect of *Glutathione S-Transferase T1* gene on melanoma risk in presence of *CDKN2A* mutations, *MC1R* variants and host-related phenotypes. *V. Chaudru[1,2], MT. Lo[1,2], F. Lesueur[3,4], K. Laud[5], H. Mohamdi[1,2], C. Marian[6], M. Barrois[3], A. Chompret[3], MF. Avril[7], F. Demenais[1,2], B. Bressac-de Paillerets[3]* 1) INSERM U794, CEPH, Paris, France; 2) Université dEvry, Evry, France; 3) Service de Génétique, IGR, Villejuif, France; 4) IARC, Lyon, France; 5) INSERM, U830, Institut Curie, Paris, France; 6) Lombardi Comprehensive Cancer Center, Washington, DC, USA; 7) AP-HP, Hôpital Cochin, Paris, France.

The effect of *CDKN2A*, the major high-risk melanoma susceptibility gene, has been shown to be modified by host-related phenotypes and variants of *MC1R* gene. The Glutathione S-transferase (*GSTs*) genes, implicated in detoxification of metabolites after UV exposure, are candidates for modulating *CDKN2A* penetrance. Few case-controls studies have investigated the effect of *GSTs* on melanoma risk, and have led to controversial results while these genes have not yet been studied in families segregating *CDKN2A* mutations. We examined the effect of *GSTP1*, *GSTM1* and *GSTT1* genotypes on melanoma risk in 25 French melanoma-prone pedigrees with *CDKN2A* mutations, in presence of *MC1R* gene variants, sun exposure, and host-related phenotypes. Logistic regression analysis, taking into account correlations among family members, was applied to 195 subjects genotyped for all investigated genes. No significant effect of null *GSTM1* allele and *GSTP1* variants (p.I105V, p.A114V) on melanoma risk was found. However, a significant protective effect of carrying at least one null *GSTT1* allele was shown: Odds-ratio (OR) adjusted for age, sex and *CDKN2A* = 0.41 (*P*=0.035) and OR adjusted for age, sex, *CDKN2A* and *MC1R* = 0.24 (*P*=0.001). Multiple logistic regression analysis showed that the factors influencing significantly melanoma risk associated with *CDKN2A* mutations were: *MC1R* and dysplastic nevi (increasing the risk) and *GSTT1* (decreasing the risk). This study demonstrates the multiplicity of genetic pathways involved in melanoma development and has important implication in melanoma risk assessment. Funded by Ligue Nationale Contre le Cancer and INCa.

Proper Analysis of Secondary Phenotype Data in Case-Control Association Studies. *D. Lin, D. Zeng* Dept Biostatistics, CB #7420, Univ North Carolina, Chapel Hill, NC.

Case-control association studies often collect extensive information on secondary phenotypes, which are quantitative or qualitative traits other than the case-control status. Exploring secondary phenotypes can yield valuable insights into biological pathways or identify genetic variants influencing phenotypes of direct interest. Indeed, recent months have seen an explosion of publications on genetic variants influencing human quantitative traits, such as height, body mass index, and lipid levels. All publications on secondary phenotypes have used standard statistical methods (e.g. least-squares estimation), which require random sampling. Because of unequal selection probabilities between cases and controls, the case-control sample is not a random sample from the general population. As a result, standard statistical analysis of secondary phenotype data can be very misleading: the estimates of genetic effects can be severely biased, the false-positive rates can be grossly inflated, and the statistical power can be substantially diminished. We present simple statistical methods that properly reflect the case-control sampling in the analysis of secondary phenotype data. The new methods provide unbiased estimation of genetic effects and accurate control of false-positive rates while maximizing statistical power. We demonstrate the pitfalls of the current methods and the advantages of the new methods in both simulated and empirical data. The relevant software is available at our website.

Method for Association Based on Hardy-Weinberg Disequilibrium. *S.-A. Bacanu, Li. Li, M. R. Nelson*
GlaxoSmithKline, Res Triangle Park, NC.

In many scenarios it is of interest to analyze markers that may deviate significantly from expectations under Hardy-Weinberg equilibrium (HWE) in study or population controls. While in cases having a disease or drug response of interest such disequilibrium can be induced for a genetic risk factor, in population or clinical controls (subsequently referred as controls) such deviations from HWE can be the result of several possible causes, including subtle stratification/admixture, active selection, genotyping errors and the possible presence of copy number variants nearby. To allow for analysis of markers in Hardy-Weinberg disequilibrium (HWD) we propose two novel HWD-based statistical tests. The first test assesses if the HWD statistic in cases is significantly increased compared to controls. The test adjusts the HWD 2 statistic in cases for the background HWD from controls. The second is a combined test of HWD and association. It computes the departures of the observed genotype frequencies in cases from the HWE frequencies estimated from controls. For both tests p-values are computed assuming the HWD 2 statistic is distributed as a non-central 2. The noncentrality parameter of the 2 distribution is estimated from the control sample and rescaled for the number of cases. We present the performance of the proposed statistical tests under a variety of scenarios involving combinations of stratified/unstratified cohorts and under the null/alternative hypothesis. The proposed tests are conservative even when analyzing markers in HWD and can increase power dramatically under many circumstances for markers in HWE. Consequently, we recommend the use of these novel tests in an exploratory analysis if not in the primary analysis itself.

Genome-wide association studies of 13 metabolic traits on the Pacific island of Kosrae suggest shared genetic architecture across populations. *J. K. Lowe[1,2,3], J. B. Maller[1,2,4], I. Pe'er[5], B. M. Neale[1,2,6], J. Salit[3], E. Kenny[3], J. L. Shea[1,2], R. Burkhardt[3], W. Ji[7], J.-N. Foo[7], R. P. Lifton[7], J. L. Breslow[3], M. Stoffel[3], M. J. Daly[1,2,9], D. M. Altshuler[1,2,9], J. M. Friedman[3,8]* 1) Broad Institute of Harvard & MIT, Cambridge, MA; 2) Massachusetts General Hospital, Boston, MA; 3) Rockefeller University, New York, NY; 4) University of Oxford, Oxford, UK; 5) Columbia University, New York, NY; 6) King's College, London, UK; 7) Yale University, New Haven, CT; 8) Howard Hughes Medical Institute; 9) Harvard Medical School, Boston, MA.

Natives to the island of Kosrae, Federated States of Micronesia, exhibit a high prevalence of metabolic disorders such as obesity and diabetes compared to Caucasian populations. A strong founder effect, severe isolation, and substantial inbreeding greatly reduced genetic diversity on Kosrae, making it an attractive cohort for mapping genetic components of complex traits. We developed methods for association analyses in this highly related population, in which 2842 subjects fall into a single extended pedigree, and performed genome-wide analyses for 13 quantitative traits. A comparison of association results for plasma lipids in Kosrae and in published studies validates our approach.

We sought to assess the added value of this non-European founder population in light of extensive GWA studies in Europeans. Using >60 published associations for quantitative traits (lipids, height, BMI, TSH) discovered in European GWA studies, we evaluate: the proportion of loci with convincingly similar effects () in Kosrae; similarity in allele frequencies and the proportion where the risk allele is too rare to evaluate in Kosrae; potential for variable LD patterns to help delimit associations; and evidence for unrelated associations in the same gene in Kosrae. Although allele frequencies are largely comparable, ~13% of loci could not be evaluated due to low frequency on Kosrae. Over 80% of the remaining loci have the same effect in Kosraens & Europeans. These data suggest strong similarity in the genetic architecture of disease between an inbred population isolate and outbred Europeans.

From laser capture micro-dissection (LCM) to high-resolution SNP-CGH microarrays: genomic architecture in two neurofibromatosis type 1-associated glomus tumors. *A. Pemov[1], C. Park[1], C. R. Lee[2], J. L. Sloan[1], D. R. Stewart[1]* 1) NHGRI/NIH, Bethesda, MD; 2) Dept. of Pathology, NCI/NIH, Bethesda, MD.

Introduction. Neurofibromatosis type 1 (NF1) is a common autosomal dominant monogenic disorder of dysregulated tissue growth. The causative gene, *NF1*, encodes the tumor suppressor neurofibromin. Glomus tumors are rare, benign tumors of the glomus body, a thermoregulatory shunt in the fingertips. Multi-focal glomus tumors in patients with NF1 have been reported, suggesting a possible association. Previously, we found somatic inactivation of *NF1* in 2 of 3 glomus tumors from a single individual with NF1. To investigate the role of other genomic perturbations in 2 NF1-associated glomus tumors, we performed comparative genomic hybridization (CGH) analysis of tumor DNA. Given the small size of glomus tumors (<10 mm in diameter) it is a challenge to separate tumor cells from normal tissue and obtain high quality DNA in the amount sufficient for downstream analysis. To succeed, we coupled LCM-based tumor cell isolation with subsequent DNA analysis on Illumina HumanHap550 SNP-CGH microarrays, a high-resolution whole genome approach requiring a low amount of input DNA. **Results.** 500 ng of tumor DNA samples were processed, in parallel with the patient's germline (lymphocyte) DNA, on the arrays. Numerous copy-neutral regions of loss of heterozygosity (LOH) were identified in both tumor samples; however, the same LOH regions were also identified in germline DNA, indicating that no tumor-specific LOHs were present in the samples. Copy number (CN) analysis revealed no large-scale (>1 Mb) deletions or duplications in the tumor samples. High-resolution CN analysis (10 Kb - 1 Mb) identified several statistically significant loci present in at least 1 tumor DNA but not germline DNA. Follow-up qPCR validation identified several candidate genes and chromosomal loci for further analysis. **Conclusions.** 1. Global genomic architecture of the glomus tumor DNA closely resembles that of the patients germline DNA. 2. Combining LCM dissection with high-resolution SNP-CGH analysis, though technically demanding, is a powerful approach for studying tumorigenesis.

Genome wide scans for signature of positive selection of noncoding regions in primates. *O. Fedrigo[1,2], R. Haygood[1,2], J. Pavisic[1], G. A. Wray[1,2]* 1) Biology Department, Duke University, Durham, NC; 2) Institute for Genome Sciences and Policy, Duke University, Durham, NC.

It has been suggested that major trait differences between humans and chimpanzees are due to changes in gene regulation rather than changes in protein structure. We previously performed a genome wide scan for positive selection of 5 flanking regions (5 kb upstream of the transcription start site), which are known to be enriched in *cis*-regulatory sequences. However, gene products can also be post-transcriptionally regulated through processes such as translation efficiency, mRNA stability and mRNA subcellular localization. This additional level of regulation is another potential target for adaptation on the human lineage. We performed a similar survey on 5' and 3' untranslated regions (UTRs) using a lineage-specific Random Effect Likelihood model comparison to test for faster evolution along the human lineage and compared the results to signatures of positive selection on 5 flanking. Our results show that the three compartments are under different selective constraints but the 5 flanking regions are more similar to the 5 UTR regions than to the 3 UTR regions, consistent with their greater similarity in regulatory function. We also contrasted the signature of positive selection with PANTHER and GO ontology classifications and expression data from Novartis Gene Expression Atlas. Different ontology categories are enriched for signatures of positive selection for each compartment. We previously found that 5 flanking region of genes involved in neural development and function and in nutrition have been targeted by positive selection. In contrast, molecular transport, signaling, development and immunity ontology categories are enriched with signature of positive selection within UTRs. The results of this study show different trends between the three regulatory compartments, suggesting that natural selection has acted differentially on these compartments for fine tuning gene products during adaptation on the human lineage.

Using mtDNA and Y-chromosome for estimating group ancestry: Implications for case-control studies. *K. Stefflova[1], M. Dulik[2], A. Pai[2], A. Walker[1], T. Schurr[2], T. Rebbeck[1]* 1) Biostatistics and Epidemiology, University of Pennsylvania, Philadelphia, PA; 2) Archeology and Anthropology.

Population-specific genetic markers inherited either biparentally (autosomal) or uniparentally (mtDNA, Y-chr.) may provide valuable information about ancestry. This genetically defined ancestry was proven to be useful in genotype-disease association studies, helping to prevent incorrect statistical inferences caused by population stratification. We examined the possible role of mtDNA and the non-recombining portion of the Y-chr. (NRY) as ancestry informative markers (AIMs) for admixed groups (self-identified African Americans (AA) or European Americans (EA)) collected as part of a prostate cancer case-control study. We deeply typed both mtDNA (HVS-I, II, 36 coding SNPs) and the NRY (37 SNPs) in a group of 226 AA cases and controls and compared this group to 206 EA cases and controls, and 49 Senegalese. Based on both mtDNA- and NRY-defined group ancestry, we confirmed that there was no statistically significant difference between cases and controls (F_{st} for mtDNA/NRY: 0.00034/0.00393 and 0.00091/0.0049 for AA and EA respectively), suggesting that our matching strategy appropriately accounted for ancestry during participant ascertainment. We then assessed admixture contributed from maternal and paternal populations. We found a sex biased admixture for AA where 13.2% of mtDNAs and 34.5% of NRYs were of non-African origin. We also found a small amount of admixture in EA (~3% mtDNA, 1.5% NRY). By typing a set of 1,509 autosomal AIMs on a subset of AAs, we confirmed that this level of admixture is reflected in the autosomal region (m_{auto} found: 19.2%6.4%, m_{auto} calculated: 23.9%). Lastly, we have used these markers for inferring the history of the Philadelphian AA using our database of published world mtDNA and NRY variation (3,800 mtDNA, 1,400 NRY) with a focus on Africans and South AAs, and stratifying it according the ethnicity, language and geography. These data paint a comprehensive picture of ancestry of the Philadelphian population and serve as a necessary control in epidemiological association studies to protect against biases in the corresponding association estimates.

Haplotype functional analysis of *ENGRAILED 2* autism risk allele. *J. Choi[1,2,4], R. Benayed[2,3,4], N. Gharani[5], L. Brzustowicz[5], J. Millonig[1,2,3]* 1) Cell and Developmental Biology, University of Medicine and Dentistry of New Jersey, Piscataway, NJ; 2) Center for Advanced Biotechnology and Medicine, Piscataway, NJ; 3) Department of Neuroscience and Cell Biology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ; 4) Graduate School of Biomedical Sciences, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ; 5) Department of Genetics, Rutgers University Piscataway, NJ.

Previous studies in the Millonig and Brzustowicz labs have determined that the homeobox transcription factor, *ENGRAILED 2 (EN2)* is a likely susceptibility gene of Autism Spectrum Disorder (ASD). The common alleles of two intronic SNPs, *rs1861972* (A/G) and *rs1861973* (C/T) are significantly associated with ASD individually and as a haplotype. Functional analyses including luciferase reporter assays and Electrophoretic Mobility Shift Assays demonstrated the associated A-C haplotype results in significantly increased expression of luciferase as well as specific binding of protein factors. These results indicate that the A-C haplotype is a functional variant contributing to increased risk for ASD. The aim of the present study was to examine whether both associated alleles contribute to this functional difference. To do this, we transfected a series of luciferase constructs into primary neuronal cultures of mouse cerebellar granule cells. The A-T and G-C haplotype constructs were tested, which have a single associated allele for either *rs1861972* or *rs1861973*. Both rare haplotypes significantly decreased gene expression disabling the A-C haplotype activator function. Oligonucleotide constructs demonstrated that each allele was sufficient for transcriptional activity but the *rs1861973* C allele modified the functional effect of the *rs1861972* A allele. These data collectively demonstrate both alleles of the associated A-C haplotype are functional. To further support the haplotype function of the risk alleles, evolutionary conservation of these alleles were investigated in primate species. The results indicate the A-C haplotype is ancestral and evolved together, implying the A-C haplotype is an important functional unit.

Association of the *CLOCK* gene to Alcohol Dependence and substance abuse in the Irish Affected Sib Pair Study of Alcohol Dependence (IASPSAD) sample. *G. Kalsi[1], P.-H. Kuo[2], F. Aliev[1], J. Alexander[1], D. G. Patterson[3], D. Walsh[4], D. M. Dick[1], C. A. Prescott[5], K. S. Kendler[1], B. P. Riley[1]* 1) Dept of Psychiatry, Virginia Commonwealth Univ, 800 East Leigh St, Richmond, VA 23298; 2) Institute of Clinical Medicine, College of Medicine, National Cheng Kung University, Taiwan; 3) Shaftsbury Square Hospital, Belfast, Northern Ireland, UK; 4) Health Research Board, Dublin, Ireland; 5) Department of Psychology, University of Southern California, Los Angeles CA, USA.

The *CLOCK gene* is a transcription factor involved in controlling chronobiological rhythmicity. Drugs of abuse can disrupt the circadian molecular mechanism essential for an organism to anticipate and adapt effectively to daily environmental cues. We examined the role of *CLOCK* gene in 575 independent cases selected from the Irish Affected Sib Pair Study of Alcohol Dependence (IASPSAD) sample and 530 controls screened for heavy drinking. Genotyping was conducted using an Illumina custom array of 1350 SNPs in candidate genes. Ten SNPs in the *CLOCK* gene were tested. The data produced 3 non-polymorphic SNPs and four showed redundancy in terms of LD information. To complete a minimum tagging set of 6 SNPs, three additional SNPs were selected using Tagger and genotyped using Taqman (ABI) assays. Association tests for single marker and haplotype analyses were conducted using Haploview v4.0. A case-only regression analysis of substance abuse symptoms as quantitative phenotype was conducted in WHAP. Single marker results reveal that 3 of the 6 tSNPs support association with AD, the most significant producing p=0.0033 (rs12649507). Multiple testing correction produced P=0.014. Haplotype analysis produced two 4-marker haplotypes; the most significant haplotype yielded p=0.003. The second haplotype demonstrates nominal significance (p=0.021). The case-only analysis using quantitative measures produced 1 tSNPs yielding nominal significance for association to substance abuse symptoms (p=0.01). Our study is the first to provide evidence for a role for *CLOCK* gene in AD and a comorbid phenotype of substance abuse. The expression of *CLOCK* gene is being explored in post-mortem brain samples.

An integrative genomics approach to biomarker discovery in breast cancer. *C. Hicks[1], R. Asfour[2], L. Miele[3]* 1) Preventive Medicine and Epidemiology & Surgery, Loyola Univ, Maywood, IL; 2) Department of Mathematics and Statistics, Loyola University, Chicago; 3) The Cardinal Bernardin Cancer Center, Loyola University Medical Center.

Recent advances in high-throughput genotyping have made it possible to conduct large-scale genome-wide association studies (GWAS)to identify gene variants associated with risk for breast cancer and a variety of other common human diseases. Over the last several years, many gene variants and genes associated with breast cancer have been identified using GWAS. However, the full breadth of the goals of high-throughput genotyping and GWAS to dissect the genetic architecture of breast cancer is rapidly running into several bottlenecks in translating findings and hypothesis from GWAS to clinical practice. One of the significant bottlenecks is the inability of GWAS to identify causal pathways and to elucidate the functions of identified gene variants and genes. This knowledge gap between gene discovery and function has significant impaired our ability to develop more effective strategies for early therapeutic intervention. In this study, we have conducted an integrative genomic analysis to identify causal pathways and to infer the causal association between gene expression and the disease. Our working hypothesis was that gene variants associated with breast cancer are mapped to multiple genes interacting within pathways, and that genes within these pathways are expressed in breast cancer subjects. We tested this hypothesis using 200 genes and 800 gene variants associated with breast cancer, derived from 59,890 breast cancer cases and 79,051 controls. We have shown through pathway analysis that genes with multiple gene variants interact within pathways. Using two publicly available gene expression data sets, we show the causal association between gene expression and the disease. In addition, we identify a novel set of genes that are regulated by genes identified from GWAS. Our analysis demonstrates that integrative genomics leveraging information from GWAS with gene expression data provides a unified approach to identification of causal pathways and modeling complex gene regulatory networks.

Sudden and premature death in adults with 22q11.2 Deletion Syndrome. *A. S. Bassett[1, 2,3], E. W. C. Chow[1,2], J. Husted[1,5], K. A. Hodgkinson[1,4], E. Oechslin[3], L. Harris[3], C. Silversides[3]* 1) Clinical Genetics Research Program, Centre for Addiction and Mental Health, Toronto, Ontario, Canada; 2) Department of Psychiatry, University of Toronto, Toronto, Ontario, Canada; 3) Toronto Congenital Cardiac Centre for Adults, University of Toronto, Peter Munk Cardiac Centre, University Health Network/Toronto General Hospital, Toronto, Ontario, Canada; 4) Department of Genetics, Memorial University of Newfoundland, St. John's, Newfoundland, Canada; 5) Department of Health Studies, University of Waterloo, Waterloo, Ontario, Canada.

Background: 22q11.2 Deletion Syndrome (22q11.2DS) is a common but under-recognized microdeletion syndrome with multisystem expression including congenital heart defects (CHD) and schizophrenia. Little is known about longevity in adults with the syndrome. Methods: We prospectively followed 264 subjects; 102 adults (> 17 years) with 22q11.2DS (44 M, 58 F; mean age 33.6 SD 10.9 years) and their 162 unaffected siblings (77 M, 85 F; mean age 36.1, SD 12.2 years). We compared survival between groups using Kaplan-Meier estimates. Findings: Twelve (11.8%; 4 M, 8 F) individuals with 22q11.2DS died at median age 41.5 (range 18.1-68.6) years, four of whom had major CHD and seven schizophrenia. No siblings died ($p<0.0001$). Six (50%) deaths were sudden, unexpected and presumed arrhythmic in origin: two in patients with complex CHD, two in patients with repaired simple congenital cardiac lesions and two in patients with no cardiac history. Survival to ages 40 and 50 years was 89.9% and 73.9%, respectively. Interpretation: Individuals with 22q11.2DS who survive childhood have diminished life expectancy and increased risk of sudden death. Further studies, including detailed post mortem examinations, are needed to facilitate research into pathophysiological mechanisms that may help identify preventive strategies. 22q11.2DS may represent another cause of sudden adult death syndrome.

Common DNA sequence variants at thirty loci contribute to polygenic dyslipidemia. *S. Kathiresan[1, 2, 3], C. Willer[4], G. Peloso[3, 5], S. Demissie[3, 5], LOLILOP, SUVIMAX, InCHIANTI, DGI, FUSION, SardiNIA, Rosetta, ISIS, METSIM, FINRISK, MDC, and FHS* 1) Program in Medical and Population Genetics, Broad Institute, Cambridge, MA; 2) Ctr for Human Genetic Research and Cardiovascular Research Ctr, Massachusetts General Hospital, Boston, MA; 3) Framingham Heart Study, Framingham, MA; 4) Ctr for Statistical Genetics, Department of Biostatistics, University of Michigan, Ann Arbor, MI; 5) Department of Biostatistics, Boston University School of Public Health, Boston, MA.

Low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol and triglycerides are quantitative risk factors for cardiovascular disease and variability in these traits is largely polygenic in origin. To map DNA sequence variants that impact blood lipoprotein concentrations, we conducted genome-wide association screens in four studies (n=11,024 from Framingham Heart Study, LOLIPOP, SUVIMAX, and InCHIANTI). We performed a meta-analysis including previously published scans [total n=19,840 composed of 11,024 new scans and 8,816 previously-published, >2.4 million single nucleotide polymorphisms (SNPs, directly genotyped and imputed)] along with replication in up to 19,826 additional individuals. We identified SNPs at 30 loci (11 newly-identified) as reproducibly associated with LDL cholesterol, HDL cholesterol, and/or triglycerides ($P<5 \times 10^{-8}$ for each locus). Newly identified common SNPs include those primarily associated with LDL cholesterol (near or in ABCG5-ABCG8, LPA, TCF1, and TIMD4); HDL cholesterol (near or in ANGPTL4, c9orf52, FADS gene cluster, HNF4A, LCAT, and PLTP); and triglycerides (near or in FADS gene cluster, PLTP, and AMAC1L2). At eight of the 30 loci, SNPs associated with blood lipoproteins were strong cis-acting regulators of gene expression in liver ($P<5 \times 10^{-8}$ for each genotype-expression association; 955 liver samples studied). The proportion of individuals with dyslipidemia (i.e. exceeding clinical cut points of high or low lipoprotein levels) increased across an allelic dosage score composed of common SNPs. These results support the hypothesis that the cumulative effect of multiple common SNPs contributes to polygenic dyslipidemia.

Complex chromosomal rearrangements detected by high-resolution whole-genome copy number analysis: implications for phenotypic variability in patients with recurrent abnormalities. *P. R. Gonzales, E. C. Thorland*
Cytogenetics Laboratory, Mayo Clinic, Rochester, MN.

Traditional cytogenetic methods have been successful at detecting abnormalities in children with mental retardation, developmental delay and multiple congenital anomalies. However, these techniques are limited in resolution and are not sufficient to determine if additional unrecognized complexity is present at the breakpoints. With the recent availability of high-resolution, whole-genome dosage analysis platforms, additional complexity is being recognized at a significant rate. A series of patients with known chromosome abnormalities was screened using the Illumina Infinium Human 1M BeadArray. Four of these cases demonstrated unexpected complexity at the site of rearrangement not appreciated with previous analyses. Case 1 had a terminal 4p deletion, consistent with Wolf-Hirschhorn syndrome. Further characterization on the 1M array revealed a 13.3 Mb terminal deletion in addition to a ~200 kb region of normal copy number and a ~600 kb duplication. Case 2 had an unbalanced 5;19 translocation, consistent with Cri-du-chat syndrome. Array analysis confirmed a 12.2 Mb terminal deletion of 5p followed by a ~600 kb duplication. Case 3 had a 20p deletion by subtelomere FISH analysis and had a chromosomal banding pattern suggestive of a complex rearrangement. Array analysis confirmed a 1.2 Mb terminal deletion of 20p followed by a ~7.3 Mb duplication. Case 4 had a cryptic 22q deletion, consistent with 22q13.3 deletion syndrome. Array analysis confirmed a 4.9 Mb terminal deletion of 22q, followed by a duplication of ~1.3 Mb. These results demonstrate that apparently simple chromosome rearrangements are often more complex at the molecular level than appreciated by traditional cytogenetic techniques. These unexpected complexities may be one factor contributing to the often considerable phenotypic variability that is observed in many of the common syndromes associated with terminal deletions and unbalanced translocations. Additional analysis is ongoing to determine the overall rate of these complex abnormalities and the mechanisms that likely contribute to these complex rearrangements.

Overloading of lipid rafts impairs SNAREs function leading to membrane traffic jam in lysosomal storage disorders. *A. Fraldi, F. Annunziata, A. Lombardi, C. Spampanato, A. Fedele, A. Ballabio* Telethon Institute of Genetics and Medicine (TIGEM), Naples, Italy.

In spite of the heterogeneity in the nature of primary storage in lysosomal storage disorders (LSDs), secondary accumulation of lipids represents a common feature of these diseases. Cholesterol and glycosphingolipids are the major constituents of lipid rafts, a liquid-ordered phase which forms a dynamic platform within cell membrane bilayers functioning in membrane trafficking and signal transduction. Lipid rafts can be isolated as detergent resistant microdomains (DRMs). An increase in the amount of DRMs and of cholesterol was observed in lysosome membranes of cells derived from a mouse model of Multiple Sulfatase Deficiency (MSD), a very severe form of LSD. As a consequence, we detected changes in protein composition/distribution within MSD lysosomal membranes associated to increased lipid rafts-mediated trapping of membrane proteins. Depletion of cholesterol from MSD lysosomal membranes reconstituted a normal protein distribution demonstrating that the abnormal pattern is cholesterol-dependent. The function of membrane proteins can be affected by their aberrant distribution. We focused on SNAREs proteins, which constitute the minimal machinery for membrane fusion. Syntaxin7 and VAMP7, two SNAREs proteins involved in the endo/lysosome fusion events, accumulate in lysosomal membrane rafts regions of MSD cells. Therefore, we hypothesized the formation of unproductive/dysfunctional complexes containing syntaxin7 and VAMP7 (and most likely other specific SNAREs) with consequent inefficient fusion process. Supporting this hypothesis, we found that while the amount of syntaxin7/VAMP7 steady-state complexes increased in MSD MEFs, the dynamics of SNAREs complex formation between VAMP7 and syntaxin7 was instead reduced, as revealed by FRAP analysis. Moreover, trapping of SNAREs proteins in rafts regions also results in inefficient re-distribution of SNAREs between cell membranes. In conclusion, we postulate that abnormal lipid storage in LSDs results in profound changes in the dynamics of endo/lysosomal membranes leading to membrane traffic jam and global lysosomal dysfunction.

Functional interactions between *cis*-regulatory SNPs involved in lactase persistence. *C. C. Babbitt[1], A. S. Swearingen[1,2], A. Ranciaro[3], S. A. Tishkoff[3,4], G. A. Wray[1,2]* 1) Institute for Genome Science & Policy, Duke University, Durham, NC; 2) Department of Biology, Duke University, Durham, NC; 3) Department of Genetics, University of Pennsylvania, Philadelphia, PA; 4) Department of Biology, University of Pennsylvania, Philadelphia, PA.

The ability to digest milk as adults (lactase persistence, LP) is a phenotype driven by distinct *cis*-acting variants in ethnically distinct human populations. Several recent studies have identified regulatory SNPs that are important for *lactase* (*LCT*) transcription. Some of these SNPs appear to have arisen independently in different human populations. Understanding how these variants interact may elucidate the functional basis of LP, as well as help to understand how this phenotype is driven by convergent genotypic changes. In order to identify the functional interactions between these *cis*-acting SNPs, we used *in vitro* constructs containing the previously identified SNPs of interest in the adjacent *MCM6* intron 13 region and a proximal *LCT* core promoter region in Caco-2 cells. Using a site-directed mutagenesis approach, we created different combinations of these functionally implicated activating SNPs. Our data show unexpected epistatic interactions between SNPs that are different between populations. Specifically, SNPs that activate transcription individually depress transcription when present together in the same haplotype. Determining the nature of interactions between polymorphisms characterized in the region with transcription factors may elucidate the mechanism by which the LP trait arose, and increase our understanding of *cis*-regulation in association with the recent adaptations to nutritional changes of *Homo sapiens*.

The factors related to attitudes toward genetic testing: Nationwide surveys on attitudes towards genome research of the public and scientists in Japan. *Z. Yamagata[1], A. Nagai[1], K. Muto[2], A. Tamakoshi[3], K. Kato[4], T. Maeda[5], I. Ishiyama[6], T. Shirai[4]* 1) Department of Health Sciences, University Yamanashi, Chuo, Japan; 2) The University of Tokyo, Tokyo, Japan; 3) Aichi Medical University, Aichi, Japan; 4) Koyoto University, Kyoto, Japan; 5) The Institute of Statistical Mathematics, Tokyo, Japan; 6) Teikyo Gakuen Junior College, Yamanashi, Japan.

Purpose: National surveys were conducted to assess attitudes toward genomic studies related to medicine in public and scientists, especially we focus on factors related to attitudes toward taking susceptibility testing with common diseases or pharmacogenomic testing. Methods: Three mail surveys were conducted; 4,000 people (age, 20-69) sampled from Japanese general population in 2005 and 3,000 people in 2008, and 2000 scientist of molecular biology and human genetics in 2007. Questionnaires include pros and cons of the promotion of genomic studies related to medicine, level of scientific literacy in genomics, demographic and socioeconomic background, and knowledge and attitudes toward genetic testing. We analyzed factors related to the attitudes toward taking genetic testing using logistic regression models. Results: More than half of the respondents are interested in taking susceptibility testing with common diseases. However, the number increases in case of pharmacogenomic testing. Scientists were more denial than the public about susceptibility testing but more supportive about pharmacogenomic testing. More people wanted to take genetic testing than 2005 in 2008 in public. Multivariate analysis revealed that taking genetic testing was related to young age, interest in the genome research, good image on genome and a high genomic literacy score.

Opportunities and obstacles of population-based genome resequencing. *X. Liu[1], T. J. Maxwell[1], L. Bull[2], K. E. Lohmueller[3], T. J. Rea[4], D. M. Muzny[2], D. A. Wheeler[2], O. Hall[2], J. S. Pankow[5], A. R. Templeton[6], A. G. Clark[3], R. A. Gibbs[2], E. Boerwinkle[1], C. F. Sing[4]* 1) Human Genetics Center, UTHSC-Houston, Houston, TX; 2) Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX; 3) Dept. of Molecular Biology and Genetics, Cornell University, Ithaca, NY; 4) Dept. of Human Genetics, University of Michigan, Ann Arbor, MI; 5) Dept. of Epidemiology, University of Minnesota, Minneapolis, MN; 6) Dept. of Biology, Washington University, St. Louis, MO.

Emerging technologies are bringing population-based DNA re-sequencing of human genomes ever closer. We have undertaken population-based resequencing of select candidate genes in a large bi-ethnic sample of 15,000 individuals. The data presented in this early abstract includes two genes, KCNJ11 and HHEX, resequenced in 1786 individuals. Introns, exons, and flanking regions being resequenced span 5.4 kb in KCNJ11 and 7.7 kb of HHEX. In this subsample, there were 85 and 148 SNPs in KCNJ11 and HHEX, respectively, with 23 and 2 non-synonymous SNPS. The proportion of rare SNPs (MAF<1%) increased disproportionately as the sample size increased, and reached 80-90%; for KCNJ11 and HHEX, respectively. The proportion of SNPs that are singletons (%s) and a test of neutrality based on singletons (as measured by D*) were influenced by sample size (n) and differed markedly between the two genes, e.g. when n=100 (or 1600), %s=32(39), D*=-1.75(-6.91) for KCNJ11; and %s=49(39), D*=-4.04(-8.47) for HHEX. This perhaps reflecting the effects of natural selection. The coverage of large sample resequencing projects is typically sparse. This has necessitated the development of methods for estimating basic population genetics parameters in the presence of sparse or missing data. For example, the estimate of Watterson's population mutation rate, , is biased downward because it overestimates the effective sample size in the presence of missing data. In summary, population-based resequencing of human genomes provides great opportunities for unveiling the role of rare variants in the genetic architecture of common disease, but novel methods are necessary to overcome the obstacles inherent in these new data.

UNDERSTANDING THE FULL SPECTRUM OF GENETIC VARIANTS2ND PHASE ENCODE RE-SEQUENCING IN 712 INDIVIDUALS FROM 10 ETHNICITIES. *F. Yu, D. Wheeler, Y. Ren, S. Scherer, D. Muzny, L. Nazareth, R. Gibbs* Human Genome Sequence Center, Baylor College of Medicine, Houston, TX.

The SNP data set characterized by phases I and II of the International HapMap Project has produced an unprecedented understanding of the linkage disequilibrium structures of the human genome, facilitated mapping loci for complex diseases, and helped elucidate signals of natural selection. HapMap studies did not capture most rare (MAF<5%) variants, which limits the potential advance in medical genetics. This large scale ENCODE re-sequencing project aims to interrogate the full spectrum of genetic variants in an unbiased manner, and enables modeling for the 1000 Genome Project. In the former ENCODE Project; ten 500kb genomic regions were directly re-sequenced in 48 samples from CEU, CHB/JPT, and YRI. SNPs were identified using automated software and validated in the HapMap samples. We set out to further extend this effort by sequencing 1Mb sequences in 712 individuals from 10 diverse ethnicities. We discovered 10,076 SNPs, among which 7,949 were novel (i.e. not present in dbSNP). We carried out quality analysis to assess a) reliability in singleton discoveries, and b) sensitivity and specificity for other variants. In the QC+ data set, the overall false positive rate is 3.2%, with the singletons having a slightly higher false positive rate of 4.3%. This data set has been released in May 2008; along with the genotyping data set obtained from 1260 HapMap individuals by Broad Institute and Sanger Institute. This is the deepest direct PCR, re-sequencing study so far, carried out on random genomic regions in control samples. It presents a tremendous opportunity for studying the population specific features, including the patterns of LD, prevalence and distribution of rare SNPs, and demographic history, in much broader geographical scope.

Metagenomic analyses of the microbial communities in cheese and their temporal variations. *D. Serre* Genomic Medicine Institute, Cleveland Clinic, Cleveland, OH.

Metagenomics is the study of genomes recovered from environmental samples. Here I use massively parallel DNA sequencing to characterize the microbial communities living in cheese, a human-made, cultural ecosystem. Cheeses display tremendous variation in appearance, pungency and palatability depending critically on the species of bacteria and molds as well as the environmental conditions used to manufacture and age fine cheeses. In addition to its economical importance, cheese production is also a concern for public health, with thousands of people becoming sick every year after eating improperly manufactured cheese. As a first attempt to describe the microbial populations and their temporal variations, I extracted DNA from cheese at four time points: immediately after insemination (day 1), at the day of sale (day 30), at the expiration date (day 90) and at day 180. I amplified short fragments of 16S and 18S ribosomal RNA genes using universal primers and obtained 100,000 sequence reads from each sample. The analysis shows that, starting from an environment relatively free of microorganisms before being inseminated with a few bacterial species, the microbial diversity increases dramatically to include multiple species belonging to several orders of bacteria and fungi. The microbial composition at the different time points reflects the changes in the environment (e.g., in pH, nutrient content and presence of oxygen) and the interactions between the different microorganisms. This study illustrates the potential of this high-throughput sequencing to study the complex microbial interactions in a changing model ecosystem.

A child with a der(Y)t(X;Y) resulting in Xp duplication and Yq deletion detected by FISH and characterized by arrayCGH. *M. Sathanoori[1], L. Gole[2], A. Goldstein[3], J. Hu[1], U. Surti[1,2]* 1) University of Pittsburgh School of Medicine, Pittsburgh, PA; 2) University of Pittsburgh, Pittsburgh, PA; 3) University of Pittsburgh Medical Center, Pittsburgh, PA.

An 11-year-old male was referred to the neurology department for follow-up due to the finding of vermis hypoplasia on prenatal ultrasound that apparently self resolved. He was noted to have autistic features, mental retardation, stuttering, and global developmental delay. No mutation was detected in the FMR1 gene. His physical examination showed many café-au-lait spots on his skin, but he lacked a second clinical feature for a possible diagnosis of neurofibromatosis 1 (NF1). A complete ophthalmology evaluation, and brain MRI was normal making NF1 unlikely. He also has IgA deficiency. Chromosome analysis detected a der(Y) with a deletion of the Yq heterochromatin region. Fluorescence in situ hybridization (FISH) showed two copies of the Xp/Yp probe, one on either end of der(Y) suggesting duplication and no signal for the Xq/Yq probe suggestive of a deletion of the Yq region. The arrayCGH results using 105K oligo array chip (SignatureGenomics, Spokane, WA), showed a copy number gain at the Xp22.33p22.2 region which includes ~2.6MB of the pseudoautosomal region (PAR) and ~13.25Mb on the non-PAR region resulting in functional partial disomy for Xp, and a copy number loss at the Yq11.221-Yqter (~11.2 Mb) resulting in partial nullisomy for Yq. The arrayCGH results confirm the cytogenetic findings and the der(Y) may be the result of an unbalanced X;Y translocation. A review of the UCSC genome browser showed that the Xp duplicated interval (~16Mb) contains at least 61 genes that include SHOX, STS, KAL1, TBL1, MID1, AMELX, and the Yq deleted interval (~11Mb) contains at least 17 genes that include the DAZ genes responsible for azoospermia or oligospermia. Partial duplications of Xp in males are rare and cases identical to our patient have not been reported. Testing on the father and the brother that is pending may give more insight into the phenotypic consequences of this chromosome abnormality.

Analysis of ABCB4 mutations in children with non-hemolytic cholelithiasis. Does development of cholelithiasis due to ABCB4 deficiency depend on hormonal load during puberty? *M. Hrebicek[1], J. Bronsky[2], M. Bouckova[1], L. Dvorakova[1], V. Valtrova[2], J. Nevoral[2], M. Jirsa[3]* 1) Institute of Inherited Metabolic Disorders, Prague , Czech Republic; 2) Pediatric Clinic, 2nd Medical Faculty, Prague, Czech Republic; 3) Institute of Clinical and Experimental Medicine,Prague, Czech Republic.

Deficiency of ABCB4 (MDR3), the phospholipid export pump of biliary membrane, results in a variety of hereditary cholestatic disorders including progressive familial intrahepatic cholestasis type 3 (PFIC-3). ABCB4 deficiency is also associated with recurrent gallbladder and intrahepatic cholesterol cholelithiasis due to low bile phospholipids before the age of 40. The development of symptoms may be conditional - episodes of cholestasis after oral contraceptives in females are characteristic. Patients with severe phenotypes carry *ABCB4* mutations on both alleles, while patients with milder and late-onset forms can only be heterozygous.

We have studied *ABCB4* mutations by direct sequencing of PCR products in a group of 23 children in whom non-hemolytic translucent cholelelithiasis developed before the age of 12. None of the patients carried *ABCB4* mutations, only known polymorphisms were found, frequency of which did not differ from the control population. At the same time we have identified heterozygosity for *ABCB4* mutations in members of 3 families with cholestatic disease and/or cholelithiasis (heterozygosity for missense or premature-stop mutations).

Hormonal load is very often the precipitating moment for symptoms of ABCB4 deficiency - such as transient cholestasis in pregnancy or cholestasis after hormonal contraceptives. We hypothesize that low (or nil) incidence of ABCB4 deficiency among pre-pubertal patients with cholesterol cholelithiasis is due to the absence of hormonal load, that leads to the presentation at later age. This is further supported by male to female ratio of 1:3 among adult patients with cholelithiasis due to ABCB4 deficiency.

Parametric Linkage Analysis in Tourette Syndrome: Analysis of A Two Generation Family with 9 Affected Individuals. *A. G. Ercan-Sencicek[1,2], C. E. Mason[2,3], D. L. Pauls[4], M. W. State[1,2]* 1) Child Study Center, Yale University, New Haven, CT; 2) Department of Genetics, Yale University, New Haven, CT; 3) Program on Neurogenetics, Yale University, New Haven, CT; 4) Psychiatric and Neurodevelopmental Genetics Unit, MGH, Harvard University, Boston, MA.

Tourette Syndrome (TS) is a developmental neuropsychiatric disorder characterized by chronic motor and phonic tics. There is substantial evidence for a complex genetic contribution to TS, but specific risk alleles have proven difficult to characterize. While the majority of gene-discovery efforts have concentrated on common variants, we have focused our attention on rare outlier individuals and families (Abelson et al 2005, State et al 2003). We have identified a 2-generation pedigree transmitting TS and related conditions in 9 affected individuals in what appears to be a Mendelian fashion. The pedigree consists of an affected father and 8 affected offspring. Given strong evidence for a shared etiology of TS, Chronic Tics (CT) and Obsessive-compulsive disorder (OCD), we assigned affected status to family members who presented with any of these three conditions. There was a history of 7 miscarriages from this parental union raising the possibility of dominant inheritance. All available family members were genotyped using the GeneChip Human Mapping 100K array. Parametric linkage analysis was conducted using the Allegro program under an autosomal dominant model. Copy number variants (CNVs) were evaluated using The Human1M BeadChip. Only a single locus was identified that approached the maximum theoretical LOD score for this pedigree of 2.1 (LOD=2.05) The result was confirmed via fine-mapping with 20 micro-satellite markers in the putative linkage interval. This 8.7 Mb region on chromosome 15q21 (D15S126 to D15S998) contains 40 brain-expressed genes. CNV analysis shows no shared structural variation among affected individuals. We are currently sequencing all candidate transcripts in the region in an effort to identify a functional mutation segregating with TS and related conditions.

Genotype to phenotype and beyond: NCBIs new resources to support evaluation of rare variants. *D. Maglott, M. DiCuccio, L. Forman Neall, M. Johnson, L. Phan, K. Pruitt, D. Shao, S. Sherry, A. Shkeda, R. Tully, M. Ward, G. Yu*
Natl Ctr Biotechnology Info, NIH/NLM, Bethesda, MD.

As sequence information becomes more readily available, it is increasingly important to be able to identify variants and interpret their significance. This presentation reviews several tools, viewers, and resources that support these needs.

Genome Workbench is an integrated application for viewing and analyzing sequence data. It can be used to align reads, identify variants, and compare them to records in dbSNP. Newly discovered variants can readily be accessioned and archived by using one of the new web-based resources that facilitate submissions to dbSNP based on HGVS nomenclature. Both single and batch methods are supported. These tools align the reference sequence in the HGVS name to the genome, identify the placement, support review by linking to other viewers including our new sequence viewer, and transform the information about the location of the variant into a submission to dbSNP. An id (ss#)is assigned to each variant, which will be clustered with other submissions of the same variant under a reference id (rs#). Attributes of each variant can also be submitted, such as links to data in OMIM, PubMed, or a pertinent web site, and frequency of observations and clinical interpretation. These enriched submissions allow display of variants and their clinical attributes that appear in a new variation viewer (VarVu), accessible from Entrez Gene, dbSNP, and OMIM. Submitters sites are also directly accessed from this viewer.

New gene-specific genomic RefSeq standards, RefSeqGene (www.ncbi.nlm.nih.gov/RefSeq/RSG) provide a stable numbering system for reporting variants on genomic sequence coordinates. These sequences are being developed in collaboration with locus-specific databases and other stakeholders Suggestions can be emailed to rsgene@ncbi.nlm.nih.gov. These resources, which are being integrated with OMIM, GeneReviews, and GeneTests, reflect NCBIs commitment to supporting clinicians and medical geneticists.

IMPACT OF LOW-COST INDIVIDUAL GENOME SEQUENCING ON GRADUATE GENETIC COUNSELING EDUCATION: AN EXPLORATORY STUDY. *A. Biser* Sarah Lawrence College, Human Genetics, Bronxville, NY.

Through the financial incentives from the public and private sectors, improvements in the cost and effectiveness of DNA sequencing technology have made the concept of low-cost individual genome scans an expected and imminent reality. Together with the continued identification and characterization of genetic factors made possible by the completion of the Human Genome Project, such individual sequencing technology could be made clinically available - used to assess ones risk for not only single gene conditions, but also common complex conditions. The use of this technology would shift the existing paradigm of clinical genetics to clinical genomics and consequently have significant implications for the education of those involved in the delivery of genetic services, namely genetic counselors. Genetic counseling training program directors and board members of the American Board of Genetic Counseling (ABGC) were surveyed to investigate if and what revisions the nations genetic counseling training programs could undergo with respect to curricular content, clinical training, and professional training to better prepare graduates for the advent of personalized genomic medicine. According to respondents, feasible modifications to the curricular content consist of putting a greater emphasis on course work in the areas of ELSI issues, common complex conditions, and health education. Clinical training would emphasize more specialty and virtual methods of counseling; while significant considerations for professional training include generating more focused efforts on professional recruitment, specialization, and variations in academic degree-level restructuring.

Shwachman-Diamond Symptoms in a Patient with Microdeletion of Chromosome Region 6p24.3 to 6p25.1. *R. Mendoza-Londono[1], L. Dupuis[1], L. Steele[2], D. J. Stavropoulos[1,2]* 1) Div. Clinical and Metabolic Genetics and; 2) Dept. Paediatric Laboratory Medicine, The Hospital for Sick Children, Toronto, ON, Canada.

Shwachman-Diamond syndrome (SDS, MIM 260400) is an autosomal recessive condition characterized by pancreatic insufficiency, hematologic defects, skeletal abnormalities and short stature. The majority of patients with the clinical phenotype have mutations in the *SBDS* gene on 7q11, but in a small proportion of cases no mutations have been found, suggesting that there is genetic heterogeneity. We describe an 8-year-old-male referred for evaluation because of history of short statute, pancreatic insufficiency, multiple infections, IgA deficiency, delayed tooth shedding, seizures and mild developmental delay. He was the product of non-consanguinous parents of English and French Canadian descent. On examination he had proportionate short stature with height and weight below the 3rd centile for age and normal head circumference. He had mild dysmorphic features. Extensive testing including chromosome analysis, cystic fibrosis testing by sweat chloride and sequencing of the *CFTR* gene, thyroid function tests and growth hormone levels were normal. Full sequencing of the *SBDS* gene did not detect any mutations. Array comparative genomic hybridization (aCGH) revealed a de-novo deletion of ~2Mb at chromosome region 6p24.3 to 6p25 involving 10 BAC clones. This genomic region harbors 7 genes including the bone morphogenetic protein 6 (*BMP6*), the gene *C6orf151*, and Neurintin 1 (*NRN1*). BMP6 belongs to the transforming growth factor beta superfamily of regulatory molecules and it is known to be expressed in multiple tissues including developing skeleton and injured brain. *C6orf151* codes for a U11/U12 small nuclear ribonucleoprotein of 48 kDa that is involved in mRNA processing and splicing. NRN1 is a neuronal protein that modulates neurite outgrowth. Our patient constitutes the first description of haploinsufficiency for genes located in this small region of chromosome 6p24. We hypothesize that one of these genes may be responsible for the Shwachman-Diamond-like symptoms present in our patient.

20p12.3 microdeletion predisposes to Wolff-Parkinson-White syndrome with variable neurocognitive deficits. *L. Potocki[1], J. V. Thakuria[4], G. F. Cox[4], X. Wang[1], W. Bi[1], M. S. Bray[1], S. W. Cheung[1], A. C. Chinault[1], B. A. Boggs[1], J. R. Lupski[1], P. Stankiewicz[1], J. A. Towbin[2], I. Hansmann[5], S. Sood[3], T. Ai[3], A. Pursley[1], X. H. Wehrens[3], J. F. Martin[6], J. W. Belmont[1], S. R. Lalani[1]* 1) Dept of Molecular and Human Genetics; 2) Dept of Cardiology; 3) Dept of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, TX; 4) Division of Genetics, Childrens Hospital Boston, Boston, MA; 5) Institut für Humangenetik und Medizinische Biologie, Halle/Saale, Germany; 6) Texas A&M System Health Science Center, Houston, TX.

Wolff-Parkinson-White syndrome (WPW) is a bypass reentrant tachycardia that results from an abnormal connection between the atria and ventricles. Mutations in PRKAG2 have been described in patients with familial WPW syndrome and hypertrophic cardiomyopathy. Based on the role of bone morphogenetic protein (BMP) signaling in the development of annulus fibrosus in mice, it has been proposed that BMP signaling through the type 1a receptor and other downstream components may play a role in preexcitation. We identified four individuals with WPW syndrome, variable dysmorphisms and neurocognitive delay and used targeted and genome-wide array comparative genomic hybridization (CGH), and candidate gene sequencing to identify the underlying genetic basis of preexcitation in these individuals. The array CGH identified non-recurrent deletions of 20p12.3, involving BMP2 in all four individuals analyzed. With the exception of one maternally inherited deletion, all occurred de novo, and the smallest of these, harbored only the BMP2 gene. In two individuals with additional features of Alagille syndrome, deletion of both JAG1 and BMP2 were identified. Deletion of this region has not been described as a copy-number variant in the Database of Genomic Variants and has not been identified in 13,321 individuals from other cohort examined by array CGH in our laboratory. Our findings demonstrate a novel genomic disorder characterized by deletion of BMP2 with variable cognitive deficits and mild dysmorphic features and show that individuals bearing microdeletions in 20p12.3 often present with WPW syndrome.

A wavelet thresholding based association test for improving adaptability. *R. Jiang, J. Dong, Y. Dai* Dept Mathematical Sci, Michigan Technological Univ, Houghton, MI.

Association studies are often used in finding susceptibility genes for complex diseases. If a single SNP has enough LD information, then methods based on a single SNP will have more power than multilocus methods. Otherwise, multilocus methods will be more powerful than single locus ones. Similarly, if the genetic information about the disease have already been captured by genotypes, using haplotype based methods will only increase the degrees of freedom and reduce their power. Otherwise haplotype based methods will provide more power to detect disease susceptibility genes. The difficulty here is to determine when we should apply multilocus methods rather than single locus ones, and for multilocus methods, when we should use genotype based methods rather than haplotype based methods. We need a method which can automatically adapt to the situation, and always be able to provide the best choice. This is accomplished by a new wavelet thresholding method that we propose in this paper. We first translate the multilocus genotype data into wavelet coefficients by discrete wavelet transform.. Then we apply thresholding on these coefficients. A coefficient is kept if its magnitude is greater than the thresholds. The scale-dependent thresholds are chosen by an empirical Bayes method. The thresholding procedure is adaptive, and it makes sure that no degrees of freedom is wasted. Finally, a score statistic is constructed using these remaining wavelet coefficients. Several association tests are compared with the proposed method. The new method has correct type I error, and it is more powerful than the existing tests under different scenarios.

ARX gene and Otahara syndrome: beyond polyalanine tract expansions. *E. Bettella[1], S. Sartori[1], L. Giordano[2], F. Darra[3], R. Polli[1], S. Russo[4], B. Dalla Bernardina[3], A. Murgia[1]* 1) Department of Pediatrics, University of Padua, Padua, Italy; 2) Department of Child and Adolescent Neuropsychiatry, Spedali Civili, Brescia, Italy; 3) Department of Child Neuropsychiatry, Policlinico G.B. Rossi, Verona, Italy; 4) Laboratory of Molecular Genetics, Istituto Auxologico Italiano, Milan, Italy.

Expansions of the first polyalanine tract of the Aristaless-related homeobox gene (ARX) protein are known to be associated with X-linked infantile spasms syndrome and epileptic dyskinetic encephalopathy. Longer expansions of the same polyalanine tract have been recently reported as a cause of early infantile epileptic encephalopathy with suppression burst pattern, also known as Otahara syndrome and a possible relationship between length of this repeat region and the severity of the epileptic phenotype has been suggested. We report three cases of Otahara syndrome: two related boys of 18 and 24 months of age, born to monozygotic twin sisters whos deceased younger brother was also described as affected by neonatal-onset epileptic encephalopathy. A third unrelated 16 year-old male subject, a sporadic case, had a clinical history of neonatal epilepsy which characteristics had evolved with time from Otahara to atypic West syndrome. His current epileptic profile is that of a Lennox-like syndrome with persistence of massive myoclonic, partial and tonic seizures; he has developed spastic tetraparesys and is cognitively severely impaired. The three patients carry two different previously unreported missense mutations of the ARX gene, both insisting on the region corresponding to the Aristaless domain, in exon 5. The two carrier mothers of the familial case, reported as completely asymptomatic, showed a balanced pattern of X-inactivation. This report further confirms the striking pleiotropy attributed to the ARX gene and demonstrates the involvement of mutations other than longer polyalanine tract expansions, in the pathogenesis of cryptogenic epileptic encephalopathy with very early onset, such as Otahara syndrome.

microRNA genomic variations in psychiatric diseases. *L. Cheng[1], J. Reid[2], O. Alvi[2], A. Lucas[2], L. Lewis[2], O. Hall[2], L. Nazareth[2], D. Wheeler[2], D. Muzny[2], D. Zhang[1], E. Gershon[1], R. Gibbs[2], C. Liu[1]* 1) Department of Psychiatry, University of Chicago, Chicago, IL; 2) Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX.

MicroRNAs (miRNAs) are short (19~25 nucleotides) single-stranded non-coding RNAs that are generated from endogenous hairpin-shaped transcripts, precursor miRNAs. miRNAs function as guide molecules in post-transcriptional gene silencing by base pairing with target mRNAs, which lead to mRNA cleavage or translational repression. With >400 members in human, miRNAs are one of the largest gene families, accounting for ~1% of the genome. Since miRNAs are abundant in brain and playing important role in brain development and function, and they can regulate the expression of many genes of neurological interest simultaneously, variants in miRNA genes have the potential to play a role in complex psychiatric diseases such as schizophrenia and bipolar disorder. We present here results on the deep resequencing of known human miRNA precursors and variants identification in 281 brain-significant miRNAs. We have sequenced the genomic DNA region of these 281 miRNAs in 282 samples including 94 HapMap (CEU, YRI, JPN and CHB) samples and 188 Stanley Medical Research Institute (SMRI) and NIMH Genetic Initiative Bipolar, Schizophrenia, Major depression and control Caucasian samples. A total of 748 SNPs have been detected, of which 103 SNPs were located in miRNA precursors. Nominally significant associations have been detected for 33 SNPs between disease/control comparison and none of these associations withstood multiple testing corrections. For those SNPs in miRNA precursors, the change in folding energy of the variant precursor were calculated and these SNPs were found tend more towards stabilizing than destabilizing the precursor. Further investigations of common and rare variant associations of the identified variants in large sample psychiatric diseases are ongoing.

Acute Intermittent Porphyria: Generation of a Knock-In Mouse Model with Biochemical and Clinical Phenotype. *M. Yasuda[1], D. F. Bishop[1], W. Edelmann[2], R. J. Desnick[1]* 1) Gen & Genomic Sci, Mount Sinai Sch Med, New York, NY; 2) Department of Cell Biology, Albert Einstein College of Medicine, Bronx, NY.

Acute Intermittent Porphyria (AIP) is an autosomal dominant disorder of heme biosynthesis, due to the half-normal activity of Hydroxymethylbilane Synthase (HMBS). Patients with AIP are prone to life-threatening, acute neurological attacks that are precipitated by various drugs, fasting, and hormonal changes. Previously, *Lindberg et al. (Nat Genet 12:195, 1996)* generated a mouse model in which phenobarbitol induction increased plasma aminolevulinic acid (ALA) and porphobilinogen (PBG), but lacked acute symptomology, presumably due to its ~30% residual HMBS activity. To investigate the pathogenesis of this hepatic encephalopathy and to develop novel treatments, efforts were directed to generate a more severely affected mouse. Mice heterozygous for R167Q and R173Q mutations, which had ~7.5% and ~1.5% of wild-type HMBS activities *in vitro*, respectively, were obtained and bred to homozygosity. R173Q mice were embryonic lethals, while homozygous R167Q mice were viable, but runted and only ~65% survived to adulthood. The mice had an early-onset profound ataxia that progressed from postnatal day (P)6-P20 and then became milder, enabling them to successfully breed. While their neurodevelopment appeared grossly normal (e.g. eye opening, ear twitch), delayed onset and impaired performance were seen for tasks requiring postural skills and complex motor coordination (e.g. rearing, running). Compared to wild-type, the mice had markedly elevated urinary ALA (~3-6 fold) and PBG (~10-30 fold), without phenobarbitol induction. The plasma uroporphyrinogen I/III ratio was increased by ~20 fold and coproporphyrinogen I was elevated by ~2 fold. Mean hepatic and erythrocyte HMBS activities were ~8% and ~17% of normals, respectively. The marked biochemical and clinical phenotype of these mice will facilitate studies of the pathogenesis of the neurotoxicity of AIP and novel therapeutics.

PCSK2 Polymorphisms Modulate the Proinsulin/Insulin Ratio and -Cell Function in an Ashkenazi Population. *J. Wasson[1], R. J. Neuman[2,3], S. Lingwei[2], G. Atzmon[4], T. Budagov[4], N. Barzilai[4], I. Blech[5], B. Glaser[5], D. Avramopoulos[6], J. A. McGrath[6], A. E. Pulver[6], M. A. Permutt[1]* 1) Department of Medicine, Washington University School of Medicine, St Louis, MO; 2) Department of Psychiatry, Washington University School of Medicine, St. Louis, MO; 3) Department of Genetics, Washington University School of Medicine, St. Louis, MO; 4) Department of Medicine (Endocrinology), Albert Einstein College of Medicine, Bronx, NY; 5) Endocrinology and Metabolism Service, Hadassah-Hebrew University Medical Center, Jerusalem, Israel; 6) Department of Psychiatry and Behavioral Sciences, Johns Hopkins School of Medicine, Baltimore.

In a case-control SNP association study of 85 selected pancreatic -cell genes, we observed highly significant association between some PCSK2 variants (7 out of 45 tested) and type two diabetes (T2D) in the Ashkenazi population. PCSK2 is responsible for the processing of proinsulin to insulin in the -cell and TCF7L2 is a transcription factor that affects the expression of PCSK2. Significant association between specific polymorphisms in TCF7L2 and T2D has been demonstrated in multiple populations, including Ashkenazi. TCF7L2 polymorphisms have been shown to modulate proinsulin levels and -cell function in a UK population. Our hypothesis is that genetic variants in these two genes affect the efficacy of proinsulin processing in the Ashkenazi which will be reflected in abnormal proinsulin/insulin ratios. The proinsulin/insulin ratio may be predictive of T2D. 1000 non-diabetic control Ashkenazi DNA samples were genotyped for SNPs rs2021786 in PCSK2 and rs7903146 in TCF7L2. 1000 corresponding Ashkenazi serum samples were assayed for proinsulin and insulin. Correlations between the SNP genotypes, quantitative traits and proinsulin/insulin ratios are now being analyzed.

Posttraumatic Stress Disorder and *SLC6A3* Haplotypes. *BZ. Yang[1,4], F. Ozbay[5], A. M. Rasmusson[6,7], H. R. Kranzler[8], J. Gelernter[1,2,3,4]* 1) Dept Psychiatry, Yale Univ Sch Medicine, West Haven, CT; 2) Genetics, Yale Univ Sch Medicine, West Haven, CT; 3) Neurobiology, Yale Univ Sch Medicine, West Haven, CT; 4) VA CT Healthcare Center, West Haven, CT; 5) Mount Sinai School Med, NY; 6) Nat'l Center for PTSD, Women's Health Science Division, VA Boston Healthcare System; 7) Boston Univ School of Med; 8) Univ CT Health Center, Farmington, CT.

Posttraumatic stress disorder (PTSD) has a heritability of about 38%. The molecular genetic basis for PTSD has been understudied compared to other psychiatric disorders, but its importance is increasingly recognized. Among the few published reports, there has been one report of association of PTSD to a variant in *SLC6A3*, which encodes the dopamine transporter protein. We collected a population-based case-control sample of 401 European-American subjects, including 182 subjects with PTSD (66% male) and 219 controls (46% male). We genotyped four *SLC6A3* markers, one each in intron 8 (M1), exon 9 (M2), intron 9 (M3) and a VNTR (M4) in the 3UTR, and conducted haplotype association analysis using score statistics adjusted by sex as a covariate. We found: (1) A significantly associated haplotype of M1-M2-M3 (global p-value = 3.46×10^{-9}), with two specific individual haplotypes being associated to PTSD. One of these haplotypes is protective (7-2-2, simulated p-value = 5×10^{-5}) with frequencies of 11.0% and 1.7% in the control and PTSD patient groups, respectively. The other is a risk haplotype (6-2-2, simulated p-value = 0.00195) with frequencies of 60.2% and 73.8% % in the control and PTSD patient groups, respectively. (2) A significantly associated haplotype of M2-M3-M4 (global p-value = 0.011), with one individual haplotype associated with phenotype (2-2-9, simulated p-value = 0.017) and frequencies of 10.3% and 15.4% in the control and PTSD patient groups, respectively. (3) Haplotype association analysis of all four markers was also significant (global p-value = 2.7×10^{-5}), with an individual haplotype (6-2-2-9, simulated p-value = 0.0015) having frequencies of 7.3% and 14.8% in the control and PTSD patient groups, respectively. We conclude that variants in SLC6A3 modulate risk for PTSD.

Association of Cleft Lip/Palate with Mutations in *FGFR2* Found in Breast Cancer Patients. *B. N. Erickson[1], K. Christensen[3], M. L. Marazita[4], J. C. Murray[1,2]* 1) Molecular and Cellular Biology, University of Iowa, Iowa City, IA; 2) Pediatrics, University of Iowa, Iowa City, IA; 3) Epidemiology, University of Southern Denmark, Odense, Denmark; 4) Oral Biology, University of Pittsburgh, Pittsburgh, PA.

Background: Understanding the lifelong clinical course of children born with congenital malformations is important to anticipate and prevent treatable causes of morbidity and mortality. Epidemiologic data has shown an increased incidence of breast cancer in women born with non-syndromic cleft with or without cleft palate (CL/P). Population studies have linked breast cancer risk to a CL/P candidate gene, *FGFR2*. **Objective:** Investigate single nucleotide polymorphisms (SNPs) in *FGFR2* for evidence of association in CL/P families. **Methods:** Seven SNPs in a highly conserved region of intron 2 in *FGFR2* were genotyped using TaqMan assays. We genotyped 221 unrelated, multiplex Filipino families (5903 individuals), 516 unrelated Danish families (2293 individuals), and 359 unrelated Iowa families (1259 individuals). Family based association testing for individual SNPs (FBAT) and haplotypes (HBAT) were used to identify association between CL/P and *FGFR2* SNPs. **Results:** FBAT analysis found no individual SNP in association with CL/P. HBAT found the CC haplotype at rs3750817 and rs2981582 is significantly associated with CL/P (p=0.0075) When a third SNP is added to the HBAT analysis (CCT haplotype at rs3750817, rs2981582 and rs7896565), significant association with CL/P was observed (p=0.0067). **Conclusions:** This study provides evidence that SNPs in *FGFR2* are associated with CL/P. Haplotypes of SNPs previously associated with breast cancer (rs3750817 and rs2981582) along with rs7896565 were significantly associated with CL/P. This work provides some evidence that SNPs in intron 2 of *FGFR2* are associated in both CL/P and breast cancer. Future work will continue to narrow in on the region of *FGFR2* association with CL/P and cellular studies may provide clues as to how CL/P relates to breast cancer risk later in life.

Visualization of multidimensional genetic likelihoods. *B. Nouanesengsy, S. Seok, V. Vieland* Battelle Center for Mathematical Medicine, The Research Institute at Nationwide Children's Hospital, Columbus, OH.

Once a linked and/or associated locus is discovered for a given disease, information on multiple genetic parameters can be gleaned via exploration of the corresponding likelihood surface. For instance, it then becomes possible to estimate parameter such as the disease allele frequency or the associated penetrances. Even more interestingly, we have previously shown that the shape of this likelihood surface contains information that can be used to distinguish linked or associated subsets of families within heterogeneous data sets, beyond the information contained in summary statistics such as the size of the maximum by-pedigree lod score. However, optimal methods for exploiting this information remain to be explored. Here we present an approach using data visualization, building on an earlier prototype (LiViT), and our PPL software package Kelvin, which can be used to output arbitrarily fine-grained grids of likelihood points across the multidimensional space (currently up to 11 parameters at a time). LiViT showed a subset of the likelihood space over a limited number of dimensions, forcing the user to repeatedly change parameters and keep a mental record of previous views. Our new approach highlights local maxima and local minima, overlapping them with level sets (points consisting of contour lines). After sampling, all points are projected to a 2D space using multidimensional scaling (MDS). MDS uses the pair-wise distances of all input points to show the structure of the data in two-dimensional space. The final points on the 2D space are used to identify geometric features such as curvature and gradient (steepness of the function) in the vicinity of critical points. We show examples of different surfaces and their 2D projections, illustrating ways in which the human eye can be used to efficiently extract genetic pattern information when high-dimensional data are displayed in appropriate fashion.

Genetic variants associated with substance dependence affect nAChRs mRNA expression. *J. Wang, C. Cruchaga, J. Budde, L. Bierut, A. Goate* Dept Psychiatry, Washington Univ, St Louis, MO.

Cigarette smoking and alcohol dependence (AD) share genetic vulnerability. Several studies reported that variants in the CHRNA5-CHRNA3-CHRNB4 gene cluster are associated with nicotine dependence (ND) and increased risk for lung cancer. We have also reported that a different group of variants within this gene cluster demonstrate association with AD. There is extensive linkage disequilibrium across the gene cluster making it difficult to determine which gene(s) affects risk for substance dependence (SD). In this study, we examine whether the variants associated with SD affect gene expression. Brain tissue from the frontal cortex of 48 unrelated, non-demented elderly European Americans were obtained from the Alzheimers Disease Research Center at Washington University. Another set of frontal cortex samples derived from 31 unrelated, non-alcoholic European Australians and 28 alcoholic European Australians were obtained from the Australian Brain Donor Program. Real-time data were analyzed using the comparative Ct method. We used ANOVA to test for evidence of differential expression in samples of different genotype. Quantitative real-time PCR analysis shows that variants associated with AD affect CHRNA5 mRNA expression but not CHRNA3 or CHRNB4. Subjects homozygous for the minor allele show 2.9-fold increase in CHRNA5 mRNA levels. Genetic variants associated with increase risk for ND do not affect mRNA expression of the genes in this cluster. The variants associated with reduced risk for ND are weakly associated with CHRNA5 mRNA levels. However, a stepwise discriminant analysis of CHRNA5 mRNA levels suggests that this association is driven by linkage disequilibrium with variants that show association with AD. In contrast, increased risk for ND is associated with a missense variant in CHRNA5 that decreases response to a nicotine agonist. The protective allele for ND does not alter the coding sequence or expression levels of any gene in the cluster. These results reveal that although variation in CHRNA5 influences risk for both AD and ND, different polymorphisms and different mechanisms of action are responsible for these effects on risk.

Fine mapping of SORL1 using memory traits to understand the genetics of Alzheimer disease. *R. Cheng[1], J. H. Lee[1], V. Santana[1], J. Williamson[1], R. Lantigua[1], M. Medrano[2], J. Jimenez[3], E. Rogaeva[4], Y. Stern[1], P.St. George-Hyslop4[4], R. Mayeux[1]* 1) Sergievsky Center/Taub Institute, Columbia Univ, New York, NY; 2) Universidad Tecnologica de Santiago, Santiago; 3) Department of Internal Medicine, Univ of Puerto, San Juan; 4) Centre for Research in Neurodegenerative Diseases, University of Toronto, Toronto, Ontario.

Neuronal sortilin-related receptor SORL1 gene has been shown to be associated with Alzheimer disease (AD) in multiple populations. To date, we and others have reported single nucleotide polymorphism (SNP) allelic and haplotype association between SORL1 and AD in multiple family, epidemiologic, and autopsy samples. We and others also have shown that suppression of SORL1 expression results in increased in A levels. If true, this relation can be extended to certain memory traits, since memory impairment precedes AD. We examined the relation between memory traits and 29 SNPs from the SORL1 gene using 1,064 family members from 210 families of Caribbean Hispanics. FBAT was used for allelic and haplotype association analysis. We found significant association for three sets of SNPs 2-4, 8-10, and 15-18, and weak associations were observed for SNPs 26 and 29. Unlike for the AD phenotype, the association with SNPs 8-10 weakened somewhat and that with SNP 13-16 strengthened. Further, the SNP association differed by memory trait. Verbal memory traits were similarly associated for the three sets of haplotypes. On the other hand, Benton recognition test revealed a different pattern of association, suggesting that this test invokes visuospatial functions and stimulates different brain parts. Here we show a systematic ways to dissect the AD and subcomponents to see how genes contribute to AD, a complex trait. In sum, our results showed that association between memory traits and some of SNPs from SORL1 gene are not uniform. However, these findings need to be replicated.

Experience with Porphyria Molecular Diagnostic Testing. *D. Doheny, I. Nazarenko, D. Kwan, K. Astrin, M. Balwani, R. J. Desnick* Dept Genetics & Genomic Scienc, Mount Sinai Sch Medicine, New York, NY.

The Mount Sinai Porphyria Molecular Testing Laboratory is a CLIA and New York State-approved laboratory for the molecular diagnosis of the porphyrias, the inborn errors of heme biosynthesis. These include the hepatic porphyrias: Acute Intermittent Porphyria (AIP; mutations (muts) in hydroxybilane synthase, HMBS), Hereditary Coproporphyria (HCP; muts in coproporphobilinogen oxidase, CPOX), and Variegate Porphyria (VP; muts in protoporphobilinogen exidase, PPOX), and the cutaneous porphyrias: Congenital Erythropoietic Porphyria (CEP; muts in uroporphyrinogen III synthase, UROS), Porphyria Cutanea Tarda (PCT) and its rare recessive form, Hepatoerythropoietic Porphyria (HEP; muts in uroporphyrinogen decarboxylase, UROD), and Erythropoietic Protoporphyria (EPP; muts in ferrochelatase, FECH). Although biochemical testing is usually diagnostic in symptomatic patients, such testing usually will not identify asymptomatic individuals. In contrast, identification of a specific mut is the gold standard for porphyria diagnosis. For each gene, all exonic, exon-intron and promoter regions are PCR-amplified and sequenced. Since 2006, our laboratory has diagnosed >100 probands and >115 affected relatives in 65 families. Among these, 64% have a hepatic porphyria (71% AIP, 15% HCP, & 14% VP) and 36% have a cutaneous porphyria (20% CEP, 37% PCT, 5% HEP, & 38% EPP). We identified 29 previously unreported mutations: in AIP, L170P, A252P, G280G, Q332X, A347P, 143delG, IVS6+1G>A; in HCP, E153V, H258R, R352C, Q165X; in VP, R168L, A467P, Y451X, IVS11+1G>C; in PCT, Q9H, P44L, D79N, G210D, H331R, R332C, 818del10, IVS10+6G>A; in HEP, V166A; & in EPP, S151F, K379X, 663delGAG, 901delTG, 1135delA. After mut database queries, >100 alleles are assessed for novel muts to rule out polymorphisms. Expression studies can be performed for missense muts to determine dysfunction. In all biochemically clearly proven patients, muts were identified. The availability of mut analysis for these porphyrias provides definitive diagnosis for patients, allowing for appropriate treatment and pre-symptomatic testing of at-risk relatives as well as prenatal testing.

Correlation of human embryo microRNA expression with infertility diagnosis. *B. R. McCallie, J. C. Parks, A. M. Janesch, M. G. Katz-Jaffe* Colorado Foundation for Fertility Research, Lone Tree, CO.

MicroRNAs (miRNAs) are short, non-coding regulatory RNAs that are an integral component in the regulation of protein expression. Loss of complete miRNA function within mice leads to embryonic lethality suggesting that miRNAs have a prominent role in development. Recent data has also pointed to the importance of key miRNAs during mouse embryo implantation. Implantation failure has been documented as a significant contributor to human infertility, thus the aberrant expression of key human embryonic miRNAs may also be associated with infertility diagnosis. To obtain insight into miRNA expression during human embryonic development, we performed real-time quantitative PCR (RT Q-PCR) on individual human blastocysts for 12 miRNAs documented to be expressed in either mouse embryos or human embryonic stem cells. We further analyzed the association of miRNA expression with human infertility diagnosis. Cryopreserved individual human blastocysts (n=18; including n=6 fertile controls; n=6 male factor infertility; n=6 polycystic ovarian syndrome) donated with patient consent and IRB approval were thawed and total RNA was isolated. Following reverse transcription, RT Q-PCR was performed in duplicates using Taqman probes. Twelve miRNA probe sets were analyzed relative to the expression of the control probe, RNU48. A miRNA expression profile of these 12 probe sets was observed in all samples. In comparison to blastocysts derived from fertile controls, statistical analysis revealed significant downregulation of 5 miRNAs in blastocysts derived from patients with male factor infertility including let-7a, let-7c, miR-21, miR-24 and miR-92 ($P < 0.05$). Blastocysts derived from patients with polycystic ovarian syndrome showed significant downregulation of 9 miRNAs including let-7a, let-7c, miR-19a, miR-19b, miR-21, miR-24, miR-34b, miR-92, miR-93 and miR-301 ($P < 0.05$). To our knowledge, this study has shown for the first time, the expression of miRNAs during human blastocyst development and the potential association with human infertility. Aberrant expression of embryonic miRNAs in correlation with infertility diagnosis could be a contributing factor to implantation failure.

Evaluation of seven CNV detection methods using whole genome SNP arrays from myopia samples. *Y. J. Li[1], A. Dellinger[1], M. Seielstad[2], L. K. Goh[3], T. L. Young[1,3], S. M. Saw[4,5]* 1) Ctr Human Gen, Duke Univ Med Ctr, Durham, NC; 2) Genome Inst. of Singapore; 3) Duke-NUS Graduate Med School, Singapore; 4) National Univ. of Singapore; 5) Singapore Eye Research Inst.

Methods for detecting copy number variants (CNVs) using genome wide single nucleotide polymorphism (SNP) arrays are relatively new and their performance is uncertain. We compared seven CNV detection methods: CBS, GLAD, CNVFinder, cnvPartition, PennCNV, QuantiSNP, and Nexus CGH. The Illumina 550K SNP array data from the Singapore Cohort study Of the Risk factors for Myopia (SCORM) were used. Calls from each analyses were compared to known CNVs in HapMap Asians and to all experimentally confirmed HapMap CNVs. Sensitivity, specificity, and kappa values were used for method comparison. Ten buccal DNA samples with genotype call rates > 99.9% were used to obtain CNVs based on two parameter settings in each method -default and optimized parameters with the highest agreement of CNV calls among samples. All methods were adjusted to generate a similar number of CNV SNPs in order to make fair comparisons among methods. The same optimized parameters for each method were applied to 13 buccal, 7 saliva, 1 blood, and 5 amplified buccal derived DNA samples from another set of 13 individuals. CNV calling using these samples was compared in order to examine the impact of different DNA sources on CNV discovery. At default parameters, CNVFinder called twice as many CNV SNPs relative to the other methods, with large variations in calls and in kappa between samples. The programs cnvPartition and PennCNV showed slightly better sensitivity and kappa than other methods under the fixed number of CNV SNPs calls. For the buccal samples, cnvPartition and PennCNV had the best overall performance with moderate numbers of CNV calls (at least 3 consecutive CNV SNPs) and good kappa values. Nexus and QuantiSNP had the highest call rates of CNVs (average 40 and 61 CNV per sample, respectively), with the highest sensitivities, but low specificity and kappa. Amplified buccal DNA resulted in higher CNV calls than other DNA sources and was unreliable. In conclusion, cnvPartition and PennCNV out-perform the other methods.

A novel mtDNA mutation, 1630A>G, in the tRNA Val gene is associated with a neuromuscular phenotype. *A. Yatsenko[1], S. Zhang[1], O. Abdul-Rahman[2], H. Zimmerman[2], A. N. Pursley[1], L. J. Wong[1]* 1) Dept MH Genetics, Baylor Col Med, Houston, TX; 2) Dept Preventive Medicine University of Mississippi Med Cent, Jackson, MS.

More than 50% of deleterious mitochondrial DNA (mtDNA) mutations are in the tRNA genes, including the most common, 3243A>G in the tRNA Leu(UUR) gene responsible for 80% of MELAS (mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes) and 8344A>G in the tRNA Lys gene responsible for MERRF (myoclonic epilepsy with ragged red fibers). However, mutations in the tRNA Val gene are rare. To date, only 3 mutations have been described to have clinical phenotype ranging from MELAS to Leigh syndrome. Here we describe a novel heteroplasmic variant, 1630A>G, in a 2-year old girl with developmental delay, short stature, midfacial hypoplasia, hypotonia with diffuse necrotizing myopathy, physical intolerance, fatigue, and muscle weakness. This variant is located at the first base-pair of the stem region adjacent to the anti-codon loop of the tRNA Val. To clarify the clinical significance of this variant, the levels of mutant heteroplasmy in various tissues including muscle, blood, urine, buccal swab, and hair follicles from the proband and probands asymptomatic matrilineal relatives were analyzed. Using allele-specific qPCR ARMS (amplification refractory mutation system), we detected 90-99% of mutant heteroplasmy in all tissues available from the proband. Interestingly, a range of ~35-70% heteroplasmy of the mutation was detected in multiple tissues of the patients asymptomatic mother. The 1630A>G variant was not detected in various tissues from the probands unaffected sibling. This variant has not been reported in 2700 individuals examined in the Polysite and Mitomap databases. The A-T pair at this corresponding position was found in 13 out of the 22 mitochondrial tRNAs. Its close vicinity to the anticodon loop makes it important in maintaining the secondary and tertiary structures of tRNA for anticodon-codon recognition and binding. This important role, and the co-segregation of high mutant heteroplasmy with the disease, suggests the1630A>G variant is pathogenic. To understand the clinical significance of the 1630A>G change, functional studies will be necessary.

Nonhomologous End Joining (NHEJ) and Fork Stalling and Template Switching (FoSTeS) as possible mechanisms responsible for the nonrecurrent *MECP2* duplications in neurodevelopmentally delayed males. *C. M. B. Carvalho[1], F. Zhang[1], P. Liu[1], A. Patel[1], T. Sahoo[1], C. A. Bacino[1], S. Peacock[1], A. Pursley[1], J. Tavyev[2], M. Ramocki[2], M. Nawara[4], E. Obersztyn[4], A. V. Morgante[5], H. Y. Zoghbi[1,2], S. W. Cheung[1], J. R. Lupski[1,2,3]* 1) Mol & Hum Gene; 2) Pediatrics, Baylor Col of Med, Houston, TX; 3) Texas Childrens Hospital, Houston, TX; 4) Med Gene, Inst of Mother and Child, Warsaw; 5) USP, Brazil.

Nonrecurrent duplications including the *MECP2* gene are one of the most common genomic rearrangements identified in neurodevelopmentally delayed males. Recently, a replication-based mechanism, Fork Stalling and Template Switching (FoSTeS), has been proposed for generating nonrecurrent rearrangements. To investigate the mechanism underlying *MECP2* duplication, we designed a 4 Mb tiling path oligonucleotide microarray that interrogates the *MECP2* genomic interval. We analyzed 28 males, each one carrying different sized duplication, varying from ~250 kb to ~2.6 Mb. Interestingly in 71% of the duplications the distal breakpoint occurred in the same 215 kb region, located 47 kb upstream to *MECP2*, a region characterized by complex architecture. In 2/28 patients, interspersed stretches of DNA of normal copy number were found within the duplicated sequence, whereas in 4/28 patients a triplicated genomic region was contained within the duplication. The presence of such complex rearrangements is characteristically observed in rearrangements generated by FoSTeS. We sequenced the breakpoints in 4 patients; 3 of them had microhomology at their breakpoint junctions suggesting the occurrence of NHEJ mechanism or, alternatively, FoSTeSX1. The fourth sample presented a complex breakpoint junction, including an inversion, suggesting that FoSTeS occurred at least 4 times to generate such a complex rearrangement (FoSTeSX4). In conclusion, we observe LCRs in the *MECP2* vicinity are probably generating an unstable non-B DNA structure, potentially facilitating a FoSTeS event during the replication process in at least 6/28 patients with complex duplications of *MECP2*.

Predictive diagnostic testing for von Hippel-Lindau Disease: Five year follow-up. *A. Rasmussen[1, 2], E. Alonso[2], A. Ochoa[2], I. De Biase[1], P. Yescas[2], A. L. Sosa[2], Y. Rodríguez[2], M. Chávez[2], S. Bidichandani[1]* 1) Dept Biochemistry, Oklahoma Univ Health Science, Oklahoma City, OK; 2) Instituto Nacional de Neurología y Neurocirugía, Mexico City, Mexico.

Von Hippel-Lindau disease (VHL) is a hereditary cancer syndrome caused by germline mutations in the VHL gene. Presymptomatic genetic testing, including children at risk, has been recommended to reduce morbidity and mortality. We tested 109 individuals from 17 families (10 definite and 7 possible VHL) for VHL mutations, 25 individuals were symptomatic and 84 asymptomatic and 43 were children under the age of 18. We identified mutations in 9/10 definite families and 3/7 possible VHL families. After delivery of the test results, the 36 mutation carriers (16 asymptomatic, 20 symptomatic) undertook an initial tumor screening, in which we identified one or more VHL tumors in 7/16 previously asymptomatic mutation-carriers. To date, these families have 5 years of follow-up. Of the 12 families where a mutation was identified, only 7 have continued surveillance (53.8%). This is in spite of very high test uptake (100% of individuals counseled). Seven mutation-carriers (3 asymptomatic and 2 children) developed a total of 26 new tumors; three died of complications. Detailed socio-demographic information for 32 adults (age 18-54y) was available. Gender, religiosity, education, income, marital status and having or not having children do not modify the likelihood of continuing the tumor surveillance (p=0.34-0.92). Adequate follow-up was also independent of pre-test anxiety or depression (p=0.38-0.76), and there was no relation with severity of the disease or number of family members affected. The only predictors of follow-up adherence were the behavior of the rest of the family, with 90.6% of the individuals being concordant regarding their surveillance decisions (p=0.038), and if the family had a female as a leader (p=0.031). Studies of long-term follow-up of patients undergoing genetic testing for hereditary cancer are needed to adapt the counseling and surveillance practices to the needs of a specific population. Doing so will result in improved treatment and quality of life for these families.

Using Free Online Genetics Information Resources for Ethics, Law, Patenting, and Policy: An Introduction to the Services of the National Information Resource on Ethics and Human Genetics--A NHGRI Project. *D. M. Goldstein, L. J. Bishop* Kennedy Institute of Ethics, Georgetown University, Washington, DC.

Attendees will learn effective search tips and strategies for using free databases and other research and reference tools from the National Information Resource on Ethics and Human Genetics. These include the GenETHX database of more than 40,000 bibliographic records (many with abstracts, tables of contents, and links to full text); the *Bioethics Thesaurus for Genetics* (a controlled searching vocabulary utilized in GenETHX since 2007); annotated bibliographies - called *Scope Notes* - on several topics; and *QuickBibs* (self-renewing database searches). In addition, information will be provided about the DNA Patent Database that tracks applications and granted patents on human dna-related discoveries; links to full text are available. This session is focused on providing an overview and orientation to support scholarship and practice in the area of ethics and human genetics and will equip attendees with ready and practical knowledge. Additional information will be shared about the free, online services of the National Reference Center for Bioethics Literature, a companion library resource at Georgetown University, which is supported by the U.S. National Library of Medicine. Presenters: Laura J. Bishop, Ph.D., Research Associate, and Doris Mueller Goldstein, M.L.S., M.A., Director, Library and Information Services, Kennedy Institute of Ethics, Georgetown University. **http://genethx.georgetown.edu** [This abstract pertains to an **ancillary meeting**.].

Analysis of genome-wide scans identifies potential novel common susceptibility loci for central obesity. *C. Lindgren[1], I. Heid[2], J. Randall[1], C. Lamina[2] for the GIANT Consortium* 1) WTCHG, University of Oxford, United Kingdom; 2) German Research Center for Environmental Health, Neuherberg, Germany.

To date, two common variants with a robust association to overall obesity predisposition have been reported (*FTO* and *MC4R*). In contrast, no specific associations have been reported to central obesity (in particular the amount of visceral/abdominal fat), which is especially relevant to metabolic disease. Separating genes influencing central adiposity (waist circumference (WC) and waist-hip ratio (WHR)) from those influencing overall adiposity (weight/body mass index (BMI)), is difficult as these traits are highly correlated (r^2~0.7). To identify common genetic variants and pathways influencing individual patterns of fat distribution and central obesity, our plan is to apply large-scale GWA-meta-analysis to widely-available anthropometric measures of central obesity looking particularly for signals with a disproportionate effect on central, rather than overall, adiposity. Such signals will then be characterised using more specific fat-distribution measures (e.g. DXA). As a first step, we have performed a meta-analysis of 13 genome-wide association scans informative for WC and WHR. In total ~2.5 million SNPs (directly-typed or imputed) were tested for association in ~28,000 individuals of European ancestry. The previously reported associations with overall adiposity of *FTO* and *MC4R* were replicated for WC ($p<5x10^{-13}$ and $p<2x10^{-8}$), but these loci were less associated to WHR ($p<5x10^{-6}$ and $p<2x10^{-4}$). In all, we identify 19 independent loci with suggestive evidence of association to WC and a further 9 to WHR ($p<5x10^{-6}$): none of these has been previously reported to be associated with overall obesity. Of these, 5 of the WC- and 9 of the WHR-signals show a disproportionate lack of evidence of association to BMI ($p>0.01$), indicating that some of these are likely to be variants influencing central adiposity, and targets for further replication and characterization using fat-distribution specific measures. Overall, our results indicate potential for common variants which influence central obesity (WC/WHR) distinct from those with an effect on overall obesity (weight/BMI) in humans.

Fragile X Mental Retardation Protein Regulates Adult Neurogenesis. *G. Shan[1], Y. Luo[2], R. Smrt[2], X. Li[2], R. Duan[1], B. Barkho[2], W. Li[1], X. Zhao[2], P. Jin[1]* 1) Dept Human Genetics, Emory Univ, Atlanta, GA; 2) Dept of Neurosciences, University of New Mexico, Albuquerque, NM.

Adult neurogenesis is regulated at multiple levels and may play a role in learning, depression, and neurodegenerative disorders. Fragile X syndrome, one of the most common forms of inherited mental retardation, is caused by the functional loss of fragile X mental retardation protein (FMRP). FMRP is a selective RNA-binding protein that forms a messenger ribonucleoprotein (mRNP) complex associating with polyribosomes. Recently, the role of Fmrp in stem cell biology has been explored in Drosophila, and Drosophila Fmrp was found to be required for the maintenance of germline stem cells. However, whether FMRP is involved in the regulation of other stem cells, particularly adult NSCs, given the mental retardation phenotype associated with fragile X syndrome, is not fully clear. Here we show that loss of Fmrp led to increased proliferation and altered fate specification of aNSCs both in vitro and in vivo. Fmrp-deficient aNSCs displayed decreased neuronal differentiation but increased glial differentiation. Furthermore, we identified specific mRNAs regulated by Fmrp in stem cell proliferation and differentiation, including GSK3, a negative regulator of -catenin and the canonical Wnt signaling pathway that has been implicated in adult neurogenesis. The loss of Fmrp resulted in reduced -catenin levels and a defective Wnt signaling pathway. Our data demonstrate that FMRP has profound regulatory roles in adult neurogenesis, providing a link between adult neurogenesis and human mental retardation.

DNA Methylation Profiling of Major Depression. *F. Haghighi[1,2], A. H. O'Donnell[3], J. R. Edwards[4], Y. Xin[1], B. Chanrion[2], P. Graham[1], V. Arango[1], A. J. Dwork[1], J. J. Mann[1,2], T. H. Bestor[3]* 1) Molecular Imaging & Neuropathology, NYSPI; 2) Psychiatry, Columbia University; 3) Genetics & Development, Columbia University; 4) Columbia Genome Center, Columbia University, New York, NY, USA.

Emerging evidence suggests that DNA methylation plays an expansive role in the central nervous system, linking this epigenetic process to neurogenesis and neuronal plasticity. Epigenetic processes may also play a key role in the etiology of neuropsychiatric disorders, possibly through abnormal genomic methylation patterns that regulate genes involved in brain development or physiology. The aim of the present study is to explore the epigenetic profile of major depressive disorder (MDD). Using experimentally and computationally validated methods, genomic DNA from the prefrontal cortex (PFC) of normal and depressed subjects is fractionated into methylated and unmethylated compartments, and then paired-end libraries are constructed followed by high-throughput sequencing via the 454 platform. To analyze and annotate these data, we developed an in-house database and a suite of analytic tools to detect potential disease-specific DNA methylation profiles. We limited our study to the PFC due to converging evidence from neuroimaging studies implicating this region in MDD pathogenesis and our own work showing that genomic methylation patterns differ markedly across regions of the human brain. We characterized the DNA methylation status of 19-26% of CpGs genome-wide for 1 normal control and 2 MDD cases. Our initial efforts focused on gene promoters where extant DNA methylation is shown to be associated with gene expression. We identified candidate genes including those of serotonergic and dopaminergic pathways, neurotransmitter release, receptor functioning, and synaptogenesis. Subsequent to bisulfite sequencing validation of these differentially methylated regions, we are expanding our analysis of these regions to a large sample of PFC tissue from 60 controls and MDD cases with comprehensive clinical and toxicological profiles. These DNA methylation abnormalities may have clinical utility as biomarkers, and evaluation of the frequency of these alterations may point to new pathways involved in MDD.

Curcumin facilitates a transitory cellular stress response in *Trembler-J* mice. *M. Khajavi[1], J. Saliba[1], G. Jackson Snipes[2], J. Lupski[1, 3, 4]* 1) Dept Molec & Human Genetics; 2) Department of Pathology; 3) Department of Pediatrics, Baylor College of Medicine, Houston, TX; 4) Texas Childrens Hospital, Houston, TX.

The molecular cause of Charcot-Marie-Tooth disease type 1 (CMT1) in the majority of patients is a duplication of a 1.4 Mb region on the short arm of chromosome 17 containing the dosage sensitive peripheral myelin protein 22 gene (*PMP22*). Alteration in *PMP22* gene expression can have profound effects on the development and maintenance of peripheral nerves. Consequently, the regulation of *PMP22* gene expression has been the focus of one therapeutic strategy for CMT1 (Sereda et al., 2003; Passage et al., 2004). These therapeutic approaches show promise in animal studies, but they are not feasible for other genetic causes of CMT because such molecular strategies only apply to *PMP22* over-expression. Many myelin gene mutants (e.g. *MPZ* and *PMP22*) cause severe disease (Dejerine-Sottas neuropathy, DSN; congenital hypomyelinating neuropathy, CHN) apparently by protein accumulation within the ER, causing Schwann cell apoptosis, and subsequently peripheral neuropathy. We have recently shown that oral administration of curcumin significantly decreases the percentage of apoptotic Schwann cells and partially mitigates the severe neuropathy phenotype of the *Trembler-J* (*TrJ*) mouse model in a dose-dependent manner. To identify specific molecular and cellular pathways through which curcumin functions in *TrJ* mice, we performed comparative expression profiling in sciatic nerves of curcumin treated and untreated *TrJ* mice. Administering curcumin induces the expression of multiple heat shock proteins including *Hsp70* while reducing ER stress sensors in sciatic nerves of *TrJ* mice. We further tested if *Hsp70* levels could influence the severity of the *TrJ* neuropathy. Notably, reduced dosage of the *Hsp70*, strongly potentiates the severity of the *TrJ* neuropathy, providing further evidence for the importance of ER associated degradation (ERAD) pathways in the pathogenesis of CMT. Together, these data provide further insights into the pathological disease mechanisms caused by myelin gene mutations and open new avenues for the use of curcumin as a therapeutic approach for ER-retained mutants.

High throughput approaches to fine mapping in regions of confirmed association. *P. Deloukas on behalf of the Wellcome Trust Case Control Consortium* Human Genetics, Wellcome Trust Sanger Institute, Cambridge, United Kingdom.

Last year we reported the identification of multiple disease loci as part of two studies: (i) a genome wide association study of seven common disease phenotypes in 14000 British Caucasian samples (2000 per disease) and (ii) a scan of non-synonymous SNPs across four additional diseases. New loci were confirmed in independent samples. However, in almost all instances the true causative variant(s) remain unknown. Acquiring the full spectrum of sequence variation across a region of association is the first step towards identification of causative variants. For a systematic analysis it is necessary to consider the entire recombination interval surrounding the focal SNP which often corresponds to several hundred kilobases of sequence. New highly parallel sequencing technologies offer the possibility to undertake such studies. In a pilot study we considered 16 loci totalling 2.7 Mb selected to be involved in multiple diseases, representing small / large regions, gene "oases" / "deserts" and regions with residual uncertainty regarding the causal gene. We defined recombination intervals for each locus; these were then PCR amplified using 5- and 10-kb amplicons in 32 CEPH individuals. PCR products for each individual were pooled and sequenced using Solexa/Illumina to a theoretical depth of 44x with the aim to obtain 20x sequence coverage. PCR coverage was on average ~90%, but fluctuated per locus, particularly in relationship to GC content. Sequence coverage correlates very well with PCR, 70-80% per locus. We identified 7024 SNPs (46% new) with the MAQ algorithm at minimum 10x coverage and 844 small indels (79% new). We obtained 99.2% concordance with SNP genotypes in HapMap - 73% of the discordant SNPs were validated in another sequenced sample. A fine mapping pilot is underway in three of the diseases (14 loci) in which 7819 SNPs are being typed across 8000 samples using the iSELECT platform. In parallel, we explore imputation-based' approaches to fine mapping in the resequenced loci. We are now proceeding to sequence loci totalling 2Mb for each disease across 80 samples representing cases and controls making start with Crohns disease.

Mitochondrial Sequence Variation has a Major Influence on the Human Transcriptome. *J. E. Curran, M. P. Johnson, J. Charlesworth, T. D. Dyer, H. H. H. Göring, E. K. Moses, J. Blangero* SW Foundation Biomedical Research, San Antonio, TX.

The mitochondria are the major site of cellular respiration and energy metabolism and appear to play a significant role in regulation gene transcription. In this study, we present the first exhaustive investigation of the influence of mitochondrial sequence variation on the human transcriptome. We have resequenced the entire mitochondrial genome using the mitoSEQr resequencing system in a sample of 384 observed maternal lineages within the San Antonio Family Heart Study (SAFHS), identifying more than 800 variants. Mitochondrial sequence was then imputed for matrilineal descendents, using the known pedigree structure, providing a total of 1,240 individuals for association analyses. Using genome-wide transcriptional profiling of lymphocyte samples from these SAFHS individuals, we measured expression in over 20,000 transcripts. For each transcript, we calculated a global test for mitochondrial genome-wide significance for association between expression levels and mitochondrial sequence variation using SOLAR. We identified over 2,200 transcripts (more than twice that expected by chance) exhibiting nominal evidence of mitochondrial regulation. Control of multiple testing across transcripts produced a set of approximately 350 transcripts that are highly likely to exhibit mitochondrial regulation. Among these are several expected cis-effects on mitochondrial transcripts such as *MTCO1* (mitochondrial genome-wide $p=4\times10^{-34}$). However, many dramatic novel trans-effects on the nuclear genome were identified including substantial mitochondrial sequence variation effects on the transcription of *CENPN* ($p=1\times10^{-70}$), *PRPH* ($p=2\times10^{-64}$), *CBX1* ($p=1\times10^{-61}$), and *TRIM65* ($p=2\times10^{-43}$) among many others. Pathway analysis revealed several functional categories in which potentially mitochondrially-regulated genes were significantly enriched including cell division ($p=3\times10^{-7}$), cell stage ($p=7\times10^{-7}$), organism survival ($p=9\times10^{-6}$), apoptosis ($p=1\times10^{-5}$), DNA binding ($p=3\times10^{-5}$), and oxidative stress ($p=3\times10^{-4}$). Overall, our data reveal a prominent role for mitochondrial sequence variation in the regulation of the human transcriptome.

A novel association between a *TNF* haplotype and serum ferritin in symptomatic hereditary haemochromatosis.

J. M. Brew[1,2], H. Rodgers[2], D. N. Azmanov[2], E. J. Walker[2], M. Bassett[3], S. R. Wilson[4], J. A. Cavanaugh[2] 1) John Curtin School of Medical Research, ANU, Canberra, Australia; 2) Medical Genetics Research Unit, ANU Medical School, Canberra, Australia; 3) ACT Health, Canberra, Australia; 4) Mathematical Sciences Institute, ANU, Canberra, Australia.

Hereditary haemochromatosis (HH) is a late onset disorder of excess iron storage that is one of the most common inherited disorders of Caucasians. The C282Y (Y) mutation and the H63D mutation in *HFE* are described in the development of ca. 85% of HH cases. However penetrance is incomplete and genetic factors in addition to *HFE* affecting iron homeostasis have been estimated to account for ca. 20-45% of the variation in iron stores in HH, but as yet no modifier genes have been identified. We studied 99 anonymous male controls and 72 YY homozygous males; 52 presented with clinical signs/symptoms consistent with haemochromatosis and 20 were asymptomatic relatives or individuals identified by incidental biochemical diagnosis. Whilst both patient groups had significantly higher mean $\log_{10}$ serum ferritin (SF) than controls ($p<0.0001$, both groups), symptomatic YY homozygotes had significantly higher pre-treatment SF than asymptomatic YY homozygotes ($p=0.0054$) - a result which has not been shown before. We then tested a reported association between the -308A promoter polymorphism in the pro-inflammatory cytokine *TNF* and serum ferritin (SF) levels and extended the analyses to include 3 other promoter SNPs, -1031, -863 and -857. A novel *TNF* 2-1-1-1 promoter haplotype (that does not include the -308A polymorphism) was associated with higher SF values in symptomatic YY individuals ($p=0.024$) with a similar trend observed in both asymptomatic patients and controls. To test whether this difference in SF could be correlated to levels of *TNF*, real-time PCR analyses were performed. Results failed to identify any differences in expression of *TNF* between samples with the 2-1-1-1 haplotype and those without. Ongoing experiments are aimed at extending the haplotype to identify regions containing elements that influence serum ferritin levels and subsequent clinical presentation.

Copy number variations (CNVs) in well-defined region of Xp22.31 are associated with syndromic phenotypes and intellectual disability. Further delineation of a new microdeletion/duplication syndrome. *G. Scharer[1], D. Manchester[1], G. Bellus[1], L. Pickler[1], M. Saenz[1], M. Raymond[1], K. McKelvie[1], M. Stillberger[1], J. March[1], B. Lund[2], M. Springer[2], K. Swisshelm[2]* 1) Dept. of Pediatrics, Div. of Clinical Genetics & Metabolism, The Children's Hospital/UCD, Aurora, CO; 2) CGL, Dept. of Pathology/UCDHSC, Denver, CO.

Syndromic and non-syndromic intellectual disability (ID) has long been linked to multiple genetic loci on the X-chromosome, while DNA microarray technology now permits the definition of the critical region of new microdeletion/duplication syndromes. Here we report for the first time overlapping symptoms in 5 patients with CNVs (1 deletion, female; 4 duplications, 2M/2F) in a well-defined region of Xp22.31 (6.8-8.2 Mb) flanked by Low-Copy Repeats as previously reported by *Wagenstaller et al.* in 2007. CNVs were detected by array based comparative hybridization (aCGH) and confirmed by fluorescent *in-situ* hybridization (FISH). All patients had various degrees of ID with moderate-severe speech delays being the most common finding. Two patients (dup) had behavioral anomalies consistent with autistic-spectrum disorder. Two patients were diagnosed with multiple congenital anomalies, one of whom carried an additional deletion on 2p21; the other had a history of multiple prenatal substance exposures. While growth was generally not affected, one patient (dup) presented with microcephaly, and another (del) had macrocephaly. Dysmorphic features were noted in every patient, including prominent forehead, broad/depressed nasal bridge with lateral nasal build-up, short palpebral fissures, low-set ears, and moderate 2-3 toe syndactyly. One patient (dup) presented with mild facial asymmetry and leg length difference causing motor delays. Neurologic exam was notable for hypotonia in all 5 patients; one patient (del) had nystagmus and mild gait ataxia. While this is a small patient sample, evidence is mounting that CNVs in a defined region of Xp22.31 are associated with distinct phenotypes of syndromic ID and patients presenting with syndromic ID should be screened for chromosomal imbalances in Xp22.31 either by aCGH or by FISH as a first line screening test.

Meta-analysis of genome-wide association data for weight in ~32000 individuals. *J. C. Randall[1], C. M. Lindgren[1], E. K. Speliotes[2], S. Li[3], GIANT Consortium* 1) WTCHG, University of Oxford, Oxford, UK; 2) Broad Institute, Harvard and MIT, Boston, MA, USA; 3) MRC Epidemiology Unit, Cambridge, UK.

Adult weight is highly heritable (~70%), but the genetic variants that predispose to it are not well known, and loci identified thus far have had modest effects. To increase power to detect such effects, we performed a meta-analysis of 13 GWA studies as part of the GIANT consortium. Populations studied are of European ancestry and constitute a total of ~32000 individuals. Weight data was stratified by gender and inverse-normal transformed. Imputation was used to obtain a set of probable genotypes for unobserved loci using the CEU HapMap reference panel and combined with directly genotyped data yielding ~2.5M SNPs in total for analysis. SNPs were tested for association in each stratum using an additive model with age as a covariate. Meta-analysis of those results was then performed for male, female, & combined subsets, and summary statistics were generated for each SNP. A T-test was used to assess the heterogeneity of the effect estimates between genders. Preliminary analysis has identified 54 independent loci with suggestive evidence of association with weight ($p5x10^{-6}$). Of these, 12 loci have previously been shown to be associated with BMI and 11 with height, including *FTO* ($p3x10^{-16}$), *HMGA2* ($p3x10^{-11}$), *MC4R* ($p3x10^{-9}$), and *GDF5* ($p4x10^{-7}$). Of the 31 remaining loci, 29 had weak association ($5x10^{-6}$p0.05) with BMI and/or height, while 2 appear to be associated with weight alone. Significant sexual dimorphism ($p1x10^{-4}$) was found in 16 out of the 54 loci. Of these 16 loci, 5 had an opposite effect direction for each gender, 3 had a significant effect in one but no effect in the other, and 8 had an effect in the same direction but of a different magnitude in each (including *MC4R*). These results are consistent with previously known associations for obesity and height, and the additional loci found to be associated in this study continue to support the hypotheses that multiple common variants influence body weight, that GWA analysis with large numbers of individuals can identify such variants, and that some variants have a sexually dimorphic effect.

The effect of chr16p11.2 microdeletions and microduplications on gene expression in Autism Spectrum Disorders. *M. Kusenda[1,2], Y. Seungtai[1], M. Wigler[1], J. Sebat[1]* 1) Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724; 2) Graduate Program in Genetics, Stony Brook University, Stony Brook, NY 11794.

Studies of genome copy number variation in Autism Spectrum Disorders (ASDs) have shown that *de novo* structural mutations play an important role in disease etiology. One of the most frequent of such mutations is a recurrent ~500 Kb deletion at 16p11.2, occurring in approximately 1% of cases, and in 0.01% of the general population. The deleted region contains more than twenty five genes, and we hypothesize that hemizygosity of one or more of these changes contributes to the autistic phenotype. Thus it is important to determine how gene function is altered by this mutation. We analyzed genome wide expression data from Epstein Barr Virus (EBV) transformed Lymphoblast cell lines, on eight families with *de novo* or inherited mutations of 16p11.2. Using RNA expression profiling by Affymetrix Human Genome U133 Plus 2.0, we examined differential gene expression in individuals with 1, 2 or 3 copies of the genomic region. Copy number variation of several genes within the region resulted in consistent expression changes (adjusted P-value < 0.005), and the most significant was aldolase A (ALDOA). This finding is interesting in light of studies that have implicated this gene in mental retardation. Data generated by this study will give insight into dosage sensitive genes within the risk variant, and may help pinpoint genes which are relevant to pathology of autism.

Regulatory genetic variants upstream of the SNCA gene. *O. Chiba-Falek[1], C. Linnertz[1], L. Saucier[1], K. Cronin[1], K. Hayden[2]* 1) Institute for Genome Sciences & Policy, Duke Univ, Durham, NC; 2) Dept of Psychiatry and Behavioral Sciences, Duke Univ, Durham, NC.

In the last decade, evidence has emerged implicating alpha-synuclein (*SNCA*) in the development of Parkinsons disease (PD), raising the critical question of what role *SNCA* plays in the molecular pathogenesis of sporadic PD. Evidence has accumulated suggesting that the role of *SNCA* in PD may be mediated through alteration of *SNCA* expression. Association studies demonstrated that genetic variability across the *SNCA* locus, including its promoter/enhancer region, is associated with susceptibility to sporadic PD. Pinpointing the biologically relevant variation within the associated *SNCA* region, however, is quite challenging, as the implicated genetic variants are in high linkage disequilibrium with each other. Thus, the causal genetic variant/s remains elusive. We hypothesized that regulation of SNCA expression levels is important in the development of sporadic PD, and, hence, regulatory variants controlling *SNCA* transcription might be PD causative. In order to explore the regulation of *SNCA* expression and to identify the regulatory genetic variant/s, we first identified genetic variations within non-coding conserved regulatory elements upstream of the *SNCA* gene. We then determined the correlation between those candidate genetic variants upstream of the *SNCA* gene and SNCA-mRNA levels in vivo in a variety of human post mortem brain tissues from unaffected individuals. Finally, we are currently confirming, in vitro, the functional consequence of the candidate genetic variants by Luciferase reporter assays. This study advances our understanding regarding the molecular mechanism modulating *SNCA* gene expression. The current evidence relating over-expression of *SNCA* to PD emphasizes the importance of this study for the identification of new therapeutic approaches for PD targeting the reduction of *SNCA* levels. Furthermore, our study serves as a general model of how to pinpoint a causal genetic variant from a set of variants with high LD scores that have been associated with complex traits and demonstrates a mechanism to elucidate the precise molecular bases of the underlying effects.

The serotonin receptor gene HTR5A affects plasma triglyceride levels in humans and mice. *Y. Zhang[1], E. M. Smith[1], L. J. Martin[2], J. Charlesworth[3], E. Moses[3], J. Blangero[3], J. Lazar[1], Z. Lazarova[1], A. H. Kissebah[1], S. Mirza[1], M. Olivier[1]* 1) Dept Physiology, Medical Col Wisconsin, Milwaukee, WI; 2) Children's Hospital, Cincinnati, OH; 3) Southwest Foundation for Biomedical Research, San Antonio, TX.

Serotonin (5-HT) has been implicated in the development of obesity, but has not been shown to regulate lipid metabolism or plasma lipid levels. One of the genes for a 5-HT receptor, HTR5A, is located in a quantitative trait locus on human chromosome 7q36 (LOD = 3.7) linked to plasma triglyceride (TG) levels in an obese cohort. Initial fine-mapping using 1000 SNPs across the 5 Mb interval uncovered association between sequence variants in the gene and TG levels. Resequencing of all exons and 3 kb of the promoter region uncovered 13 SNPs within the 20 kb gene interval. Association analysis demonstrated that four of these SNPs were associated with TG levels ($p<0.002$). Subsequent BQTN analysis identified a SNP (rs3734967) with substantial posterior probability ($p=0.59$), suggesting that this variant may be causal. This SNP, which is 2kb upstream in the promoter, accounts for ~5% of the observed linkage in the QTL region in our cohort. Each copy of the G allele, on average, increases TG levels by 8-9%. Since HTR5A is exclusively expressed in the brain, we tested the effect of the two alleles of rs3734967 on transcription factor binding using two human glioma cell lines in gel-shift assays. We detected significantly altered binding of nuclear proteins to the A allele when compared to the G allele of the variant. Mass spectral analysis revealed differential binding of multiple DNA binding proteins including transcription activators (STAT1 and STAT3) and DNA helicases. Finally, plasma TG levels of htr5r KO mice (B6.129-Htr5atm1Dgen) are significantly lower ($p=5\times10\text{-}4$) when compared to heterozygous littermates at 6 wks of age, suggesting that HTR5A affects plasma TG levels in both humans and mice. These results, for the first time, implicate serotonin in obesity-related lipid homeostasis, and may allow the development of alternative pharmacological lipid-lowering therapies to prevent the cardiovascular complications of obesity.

Large-scale investigation of xenobiotic metabolism candidate genes and related pathways in amyotrophic lateral sclerosis(ALS) identifies associations in KLHL1 and VIL2. *E. Rampersaud[1], Y. Yang[2], W. Chen[2], M. Saeed[1], H. Khan[2], J. Armstrong[2], M. Zheng[2], D. Ma[1], R. Sufit[2], S. Heller[2], Y. Jianhua[2], N. Siddique[2], J. L. Haines[3], M. A. Pericak-Vance[1], T. Siddique[2]* 1) Miami Inst. Human Genomic, University of Miami; 2) Department of Neurology, Northwestern University; 3) Center for Human Genetics Research, Vanderbilt University.

Amyotrophic lateral sclerosis(ALS) is a fatal neurodegenerative disorder for which there are no known preventative treatments.It has long been suspected that toxicity from exposure to xenobiotic (natural or artificial substances foreign to the body) and endogenous compounds plays a role in the etiology of sporadic ALS (SALS).However, it is unclear whether genetic variability modifies the level of toxicity of such compounds.We performed the largest association analysis to date of 5,266 SNPs in 1,366 genes involved in xenobiotic metabolism and related pathways in 343 unrelated individuals with SALS, 368 neurologically normal unrelated controls and 195 singleton trio families.The genotype callrate of these SNPs was 95% the average MAF was 0.30.No evidence of population stratification was detected.SNPs were tested for departure from Hardy Weinberg Equilibrium (HWE) and association analyses in the combined dataset of cases, controls and trios was carried out with the UNPHASED software.Three common correlated SNPs (rs7985162,rs2161760,rs1424313;r^2>0.9)in intron 6 of the Kelch-like 1 gene (KLHL1) on chr13 were the most strongly associated with SALS (P~10^{-6}, P=0.025 after stringent Bonferroni correction testing for all 5,266 SNPs).Also of interest were 5 SNPs (rs7760064,rs2306746, rs3102990,rs7754951, rs3127196,P<0.001)in intron 9 in the Ezrin gene (VIL2) on chr6.In an independent study of SALS in British and German samples, rs2171209 in VIL2 was associated with SALS (genotypic P=0.0048) and this SNP is in moderate LD (r^2>0.30) with 3 of the VIL2 SNPs associated with SALS in our study (rs2306746, rs3102990,rs3127196,P<0.001).The significance of our findings for KIHL1 and the confirmation of our results for VIL2 strongly implicate these genes, and further indicate that genetic susceptibility in genes related to toxin exposures increases SALS risk.

Joint Analysis of Germline p53 Mutation, MDM2 SNP309, and Gender on Cancer Risk in Family Studies of Li-Fraumeni Syndrome. *C. C. Wu[1], R. Krahe[2], G. Lozano[2], S. Shete[1], C. I. Amos[1], L. C. Strong[2]* 1) Dept Epidemiology, Univ Texas MD Anderson CA Ctr, Houston, TX; 2) Dept Genetics, Univ Texas MD Anderson CA Ctr, Houston, TX.

Li-Fraumeni syndrome (LFS) is a rare familial cancer syndrome characterized by a high frequency of early-onset and diverse tumor types and an increased frequency of multiple primary tumors. Germline p53 mutations have been identified in most LFS families. Recently, a high-frequent genetic variant of single-nucleotide polymorphism (SNP) 309 in the MDM2 gene, a direct negative regulator of p53, was shown to be a modifier of cancer risk in p53 mutation carriers. Independent reports showed a significantly earlier age of cancer onset for the SNP309 G-allele carriers than homozygous T-allele carriers by 7 to 15 years on average. However, mathematical modeling of family studies allowing for multivariate interactions between p53 mutation, SNP309, and gender on cancer risk has not been explored. We propose the use of joint segregation and linkage analysis on the basis of Cox proportional hazards regression to assess joint effects of p53 mutation, SNP309, and gender on age of cancer onset. We first analyzed 6 extended LFS pedigrees ascertained through childhood sarcoma patients with germline p53 mutations and included all invasive cancers, except non-melanoma skin cancer and in situ carcinoma, as a single combined phenotype. Using p53 as a linked marker in the analysis, our results showed strong evidence of linkage disequilibrium (LD) between the putative trait locus and p53 locus. The LOD-Score difference of 1.5 is considered evidence for a better model. In our analysis, the highest LOD-Score for the model with LD is higher by 3.07 than that for the corresponding model without LD, suggesting a far better fit. A bigger sample comprising 22 extended kindreds with p53 germline mutations ascertained through clinical LFS phenotype is currently available for analysis. This sample includes 133 p53 mutation carriers with MDM2 SNP309 genotypes. Currently, we are quantitatively assessing the marginal and joint effects of germline p53 mutations, MDM2 SNP309 and gender on age of cancer onset in these 22 pedigrees.

Two New Mutations in FBN 1 Gene Associated With Marfan Syndrome in Puertorrican Population. *N. Ramirez[1], A. S. Cornier[2,5], J. Flynn[1], C. Burgos[2], N. Arciniegas[3], S. Carlo[2,4]* 1) La Concepcion Hospital, Dept. of Orthopedics, San German, Puerto Rico; 2) La Concepcion Hospital, Molecular Medicine Dept.,San German, Puerto Rico; 3) La Concepcion Hospital, Dept. of Pediatrics, San German, Puerto Rico; 4) Ponce School of Medicine, Dept. of Biochemistry, Ponce, PR; 5) Univ. of Puerto Rico School of Medicine, Dept. of Internal Medicine, Rio Piedras, PR.

Marfan syndrome is a connective tissue disorder with clinical variability. It mainly involves the ocular, skeletal, and cardiovascular systems. Mutations in the FBN1 gene and TGFBR2 gene had been associated with wide phenotypic variability in Marfan Syndrome, ranging from isolated clinical manifestations to severe and rapidly progressive disease. Puerto Rico is a small island in the Caribbean with unknown prevalence and incidence of Marfan Syndrome. Several Patients are diagnosed in the genetic clinics. We are reporting two new mutations associated with clinical diagnosis of Marfan in Puertorrican population. Molecular genetic testing of the FBN 1 gene detects 70-90% of mutations and is available in clinical laboratories.DNA sequencing of the FBN 1 gene had revealed two novel sequence variants: Heterozygous 6515_6516delTTinsG in exon 53 results in a premature stop codon (V2172fsX12), and 4505G>A (C1502Y) in exon 36. These mutations have never been reported before. Marfan findings in these patients includes dilatation of the aorta, mitral valve prolapse and triscuspid valve prolapse.Other features inludes ectopia lentis, lens dislocation and specific skeletal features. Amongs the skeletal system involvement presented with bone overgrowth and joint laxity and the extremities are disproportionately long for the size of the trunk (dolichostenomelia). Overgrowth of the ribs can cause pectus excavatum or pectus carinatum were present. Minimal Scoliosis was found in both patients.

Recurrent translocation t(4;11)(p16.2;p15.4) mediated by homologous low-copy repeats in three families with autistic spectrum, multiple congenital anomalies, and dysmorphic features. *Z. Ou[1], P. Stankiewicz[1], A. Patel[1], T. Sahoo[1], AC. Chinault[1], R. Shippy[2], J. Collins[2], JR. Lupski[1], SW. Cheung[1]* 1) Dept Molec & Human Gen, Baylor Col Med, Houston, TX; 2) Affymetrix, Santa Clara, CA.

To date, only two recurrent constitutional non-Robertsonian translocations have been described. The most common translocation t(11;22)(q23.3;q11.21) is mediated by AT-rich cruciform structures and the t(4;8)(p16.2;p23.1) translocation is mediated by the olfactory receptor-gene cluster low-copy repeats (LCRs). We describe three unrelated families with an unbalanced translocation der(4)t(4;11)(p16.2;p15.4) identified by targeted array CGH (CMA V5, V6.1, and V6.2) and verified by FISH. The patients phenotypes include autistic spectrum, multiple congenital anomalies, and dysmorphic features. Using SNP arrays (Affymetrix 6.0), the breakpoints were mapped at 3.55 Mb, 3.58 Mb, and 3.4 Mb in 11p15.4, and at 4.15 Mb, 3.85 Mb, and 3.9 Mb in 4p16.2, respectively. Sequence analysis of the breakpoint regions revealed the presence of large (~300 kb) LCRs of 94-98% interchromosomal sequence identity; the translocation breakpoints mapped within homologous subunits and thus were mediated by these LCRs. Interestingly, a similar paternally inherited translocation t(4;11) with breakpoints mapping at 3.4 Mb in 11p15.4 and at 4.6 Mb in 4p16.2 has been reported by Russo et al. (2006) in a patient with features of both Wolf-Hirschhorn and Beckwith-Wiedemann syndromes. In addition, we identified a pericentric inversion inv(11)(p15.4q25), and using CMA V6.1 and the SNP arrays defined the breakpoints within the same LCR in 11p15.4. The other breakpoint was located in a LCR-free region. Our data indicate that the LCR in 11p15.4 is a novel genomic instability region that can mediate the third recurrent constitutional non-Robertsonian translocation t(4;11)(4p16.2;11p15.4) and can stimulate the formation of other chromosomal aberrations in humans. The frequency of this NAHR mediated rearrangement compared to the other two reported recurrent translocation awaits single sperm PCR analysis of the recombination event and further chromosomal studies of patients with the resulting imbalances.

Autosomal Dominant Large Fontanelles - Prenatal Diagnosis and Postnatal Outcome. Report on Two Families.

M. Szak[1], N. Martin[1], A. Toi[2], D. Chitayat[1] 1) Prenatal Diagnosis, Mount Sinai Hospital, Toronto, ON, Canada; 2) Dept. of Diagnostic Imaging, Mount Sinai Hospital, Toronto, ON, Canada.

Very large anterior fontanelles are known to be associated with a variety of conditions and can be misinterpreted as acrania on fetal ultrasound. Obtaining detailed family history and parental examination to rule out cleidocranial dysplasia (CCD) are of utmost importance for accurate diagnosis. We report two families with a history of very large and delayed closure of the anterior fontanelles that is consistent with an Autosomal dominant mode of inheritance and diagnosed through the prenatal finding of a very large anterior fontanelle on 2D and 3D ultrasounds. Family 1: Our patient was a G3P2L2 mother. Fetal ultrasound showed absent cranial bone ossification at 13.1 weeks gestation. Fetal ultrasound at 16 weeks gestation showed a prominent anterior fontanelle measuring 1.9cm. Family history was significant for very large anterior fontanelles and splayed sagittal sutures detected on the father, the couples son and the paternal grandmother. Physical examination of the father showed OFC2 SD, height at 25-50th percentile, and slopped shoulders. Although his features were not consistent with CCD we performed molecular analysis for the RUNX2 gene on the father and no mutation was detected. The pregnancy is ongoing. Family 2: The patient was born to a G3P2SA1L2 mother. Fetal ultrasound at 18.5 weeks gestation revealed an absent/hypoplastic nasal bone, a large anterior fontanelle and bilateral 5th finger clinodactyly. Delivery was at 38.5 weeks gestation via a planned repeat c-section. Physical examination at 6 months of age revealed macrocrania and a very large anterior fontanelle. Detailed family history revealed that father was also born with a very large anterior fontanelle that eventually closed at 3 years of age. He has no findings consistent with CCD. To the best of our knowledge these families present a novel condition with large anterior fontanelle and absent/hypoplastic nasal bone, which was detected prenatally and confirmed postnatally. Making the diagnosis is of utmost importance to avoid false diagnosis of acrania and to lower parental anxiety.

Linking Collagen Genotypes to Clinical Phenotypes. *D. Bodian[1], M. Balaraman[2], B. Brodsky[2], T. Klein[1]* 1) Genetics Dept, Stanford University, Stanford, CA; 2) Department of Biochemistry, University of Medicine and Dentistry of New Jersey-Robert Wood Johnson Medical School, Piscataway, NJ.

Type I collagen is the major protein in bone, skin, tendon, sclera, and blood vessels and is responsible for the structural integrity of these tissues. The most common disease associated with mutations in type I collagen is osteogenesis imperfecta (OI), or brittle bone disease. OI presents in a wide range of clinical severities and the relationship between genotypes, molecular phenotypes, and clinical phenotypes is poorly understood. Although it is known that the phenotype depends on the nature of the mutation and its location with the gene, it is not currently possible to reliably predict the disease severity from the mutation. The ability to make such a prediction to guide genetic counseling decisions is becoming critical as diagnostic strategies move to direct sequencing of the genes. We are developing a computational method to predict the lethality of OI-associated collagen mutations using a divide-and-conquer approach. Mutations likely to share a mechanism of lethality were identified and each set was modeled separately. Models were validated both computationally, using independent test sets of mutations, and experimentally. The computational and experimental results together lend insight into the effect of mutations on the structure and function of collagen and the etiology of OI.

RNAi knockdown reveals an important role for glycerol kinase genes in development and metabolism of *Drosophila*. *P. J. Wightman[1], G. R. Jackson[2], K. D. Dipple[1,3]* 1) Department of Human Genetics, David Geffin School of Medicine at UCLA, Los Angeles, CA; 2) Department of Neurology, David Geffin School of Medicine at UCLA, Los Angeles, CA; 3) Mattel Children's Hospital at UCLA, Los Angeles, CA.

In humans, glycerol kinase deficiency [GKD; MIM 307030] can be asymptomatic or symptomatic ranging from mild to severe phenotypes. The lack of genotype/phenotype correlations in GKD patients however has led us to hypothesize the existence of modifier loci. We have created a *Drosophila* model for GKD using the UAS/GAL4 system for RNAi-mediated knockdown of the two closest *Drosophila* homologues (CG7995 and CG18374) to the human glycerol kinase gene (*GK*, Xp21). Using ubiquitous, fat body and muscle GAL4 drivers, RNAi knockdown of CG7995 and CG18374 identified phenotypes ranging from larval lethality, pupal lethality, adults with wing abnormalities to adults with shortened life span. Lines that survived to adulthood died at a rate similar to starvation when placed on a glycerol-only food source. This inability to metabolize glycerol was observed for knockdowns of both *GK* homologues, indicating that both are essential for normal glycerol metabolism. As expected, elevated glycerol levels and decreased glycerol kinase transcript levels were detected in multiple knockdown lines that survived to adulthood. Pupal lethality of RNAi lines using a fat body driver was rescued using transgenic lines for both *GK* homologues. These rescued flies had shortened lifespan, indicating that normal *GK* expression levels are necessary for normal lifespan. Thus, we demonstrate a genetically tractable model of GKD that can be used to screen for modifier loci.

Localization of a Dominant Genetic Susceptibility Factor in Familial Malignant Mesothelioma. *J. E. Below[1], A. Pluzhnikov[1], K. Aquino-Michaels[2], M. Nasu[2], V. Paz[1], B. Mossman[3], H. Pass[4], J. R. Testa[5], M. Carbone[2], N. J. Cox[1]* 1) University of Chicago, Chicago, IL; 2) University of Hawaii, Honolulu, Hawaii; 3) University of Vermont, Burlington, VT; 4) New York University, New York, NY; 5) Fox Chase Cancer Center, Philadelphia, PA.

Malignant mesothelioma (MM) is the primary, yet rare cancer of the pleura, and is generally found in populations with asbestos exposure1. The prognosis of MM patients is poor due in part to a prolonged latency period (frequently greater than 25 years) from initial exposure to clinical diagnosis1, and the highly malignant nature of the cancer. Upon diagnosis the median survival time is 10 months2, and early detection remains the best approach for reducing morbidity and mortality. An American family with no known asbestos exposure, but a markedly high rate of MM has been identified. All available family members have been genotyped using the Affymetrix Genome-Wide Human SNP Array 6.0. Prior to linkage analyses, familial relationships were checked and corrected using PLINK3. Parametric linkage analyses based on a 0.2 cM SNP map assume a rare dominant model with age-dependent liability classes modeling the expected change in penetrance for different age groups. Preliminary findings indicate that a region on chromosome 6 shows genome-wide significance for linkage (LOD score >3.4). We report details of these analyses, and the results of sequencing and copy number analysis in the disease-linked region on chromosome 6. 1. Testa, J.R. & Jhanwar, S.C. Textbook of pleural diseases, 120-130 (Arnold, London, New York, 2003). 2. Emri, S. et al. Prognostic significance of flow cytometric DNA analysis in patients with malignant pleural mesothelioma. Lung Cancer 33, 109-14 (2001). 3. Purcell, S. PLINK v0.991. (2007).

Generation of a BAC transgenic mouse model for FXTAS. *Y. Li, P. Jin* Dept Human Genetics, Emory Univ, Atlanta, GA.

Fragile X-associated tremor/ataxia syndrome (FXTAS) is a progressive neurodegenerative disorder recognized in fragile X premutation carriers. Using Fruit fly, we have previously demonstrated that elongated noncoding CGG repeats in FMR1 allele as the pathogenic cause of FXTAS. To further develop robust FXTAS mouse model and study the molecular basis of rCGG-mediated neurodegeneration, we have used two-step BAC modification protocol with PLSD53SC-AB shuttle vector to modify a BAC containing the full length of calbindin genomic sequence by which the transgene expression would be Purkinje cell-specific. The precise manipulation resulted in the replacement of Calbindin exons by transgene containing EGFP coding region with (CGG)90 in the 5-UTR in the modified BAC bone. The modified BAC has been used to generate transgenic mice and could express the (CGG)90-EGFP specifically in mouse Purkinje cells driven by regulatory elements from mouse endogenous Calbindin gene independent of chromosomal location of the transgenic BAC, while the endogenous FMRP1 levels are not affected with overexpression of rCGG repeats. Development of this mouse model will provide new tool to further understand the molecular basis of FXTAS.

High population attributable risk (PAR): the PAR-adox of genome-based personalized medicine. *A. C. J. W. Janssens, C. M. van Duijn* Dept Epidemiology, Erasmus MC, Rotterdam, Netherlands.

Population attributable risk (PAR) is increasingly used in genetic association studies to demonstrate the population health impact of a genetic susceptibility gene and of combinations of multiple susceptibility genes (genetic profiles). PAR is the percentage of cases that is attributable to a risk factor, and hence the percentage of cases that can be prevented when the adverse effects of the risk factor are eliminated. Several alternative formulas are available for the calculation of PAR, but the simplest ones, assuming independent effects of susceptibility genes, are generally used. The properties of the PAR for evaluating genetic profiles are not well understood. Our studies of the performance of the PAR for the evaluation of genomic profiles show three important characteristics. First, the PAR estimates easily approach 100% when multiple genes are combined, even when the heritability of disease is low. This is not explained by the risks associated to the profile but by the joint frequency distribution of the risk alleles. Second, our evaluation suggests that a high PAR often comes with low predictive value when the effects of single genes are small. This discrepancy is explained by an inflation of the odds ratios of genetic profiles when rare profiles are used as baseline. Third, our study suggests that a high PAR of genetic profiles in complex diseases often implies that genetic screening is NOT efficient but rather asks for a population-based approach targeting the full population rather than genetically determined high-risk groups. In sum, we show that the PAR is very poorly related to the predictive value of a genetic test and that a high PAR does not imply that a genetic test will be useful.

Specific telomere length changes in length in chronic myeloid leukemia. *O. Samassekou[1], J. Herbert[2], A. Ntwary[1], B. Xiong[1], E. Bouchard[1], CC. Grenier[3], J. Y.[1]* 1) Dept Medical Genetics, Sherbrooke Univ (CHUS), Sherbrooke, PQ, Canada; 2) Banque des cellules leucémiques, Montréal, PQ, Canada; 3) Dept pédiatrie, Sherbrooke Univ (CHUS), Sherbrooke, PQ, Canada.

Chronic myeloid leukemia (CML) is a neoplasia characterized by a proliferation of a myeloid cell lineage and a translocation t(9;22). As most of the cancers, the telomere length in CML cells is shorter than normal blood cells. However, currently no data are available about the specific telomere length in CML. We studied the telomere length on each chromosome. After obtaining metaphases from CML cells, in situ hybridization with peptide nucleic acid probes was performed. The telomere length of each chromosome arm was measured according to the fluorescence intensity. We surprisingly noticed a lengthening of the telomere on the short arm of a chromosome X. This lengthening was confirmed by telomere restriction fragment technique. In addition, we showed a shortening of the telomere on the long arm of a chromosome 21. We measured the telomerase activity by real time polymerase chain reaction. We found an increase of telomerase activity in all samples. However, this augmentation could not explain the huge difference between short and long telomeres on one hand, and between the homologous chromosomes on the other hand. Then, we studied sister telomere exchange and found an increase of the exchange events in leukemia cells. Besides, the action of the telomerase to maintain telomere length, the alternative lengthening telomere (ALT) might be another mechanism to conserve telomere length in CML. It is important to take into account together the two mechanisms maintaining telomere length when targeting the telomerase by therapeutic agents because the telomerase activity can hide the ALT mechanism. The telomere change in length associated with specific chromosomes could be used as a marker for the follow up of patients and serve for a better understanding of telomere biology.

TP53 R72P polymorphism and childhood leukemia susceptibility. *T. Do, E. Ucisik-Akkaya, C. Davis, B. Morrison, M. T. Dorak* Genomic Immuoepidemiology, HUMIGEN LLC, the Institute for Genetic Immunology, 2439 Kuser Rd., Hamilton, NJ.

Genomic and immunologic surveillance mechanisms are crucial in protection from cancer. TP53 is one of the major regulators of genome surveillance. Inherited TP53 mutations cause familial cancer. Among the natural sequence variants of TP53, the exon 4 SNP rs1042522 (R72P) has been reported to modify the risk for solid tumors. To investigate its relevance in childhood acute lymphoblastic leukemia (ALL) susceptibility, we genotyped 114 cases with childhood ALL and 389 newborn controls from Wales (UK). Homozygosity for R72P showed increased risk for childhood ALL with no sex specificity (OR = 2.9, 95% CI = 1.5 to 5.6; P = 0.002). The additive model confirmed the gene-dosage effect (P = 0.002). TP53 association was independent from other associations found in the same sample, in particular the HLA complex and NKG2D associations. We then examined the intron 1 polymorphism known as SNP309 (rs2279744) within the MDM2 gene, which encodes a negative regulator of TP53. Although MDM2 SNP309 is associated with adult cancer risk with sex effect, it did not show any association with childhood ALL, even after stratification by sex. There was no statistical interaction between MDM2 and TP53 SNPs except that the risk conferred by TP53 R72P homozygosity was greater for girls if they had the wildtype MDM2 genotype (OR = 7.1; P = 0.02) as opposed to the variant-positive genotype (OR = 1.4). We also genotyped 12 SNPs in HLA complex genes whose products interact with TP53 and/or belong to genomic surveillance/apoptosis pathways (DDR1, IER3, LTA, BAT3, DAXX). No interaction test reached statistical significance for the HLA complex variants and TP53 R72P. Only the 5 end haplotype tagging SNP of DAXX (rs2073524) showed a suggestive interaction (P = 0.05). In females, the risk association with TP53 R72P homozygosity got stronger in the presence of homozygosity for the DAXX variant allele (OR = 12.6, P = 0.001). Our results suggested that TP53 R72P polymorphism is one of the stronger modifiers of risk for childhood ALL susceptibility and might interact with its regulator MDM2 in females.

A two-stage analysis using Random Forests and association testing identifies novel epistatic relationships with *PTPN22* in rheumatoid arthritis. *P. P. Ramsay[1], F. B. S. Briggs[1], E. Madden[1], M. F. Seldin[2], P. K. Gregersen[3], L. A. Criswell[4], L. F. Barcellos[1]* 1) Univ of California, Berkeley; 2) Univ of California, Davis; 3) The Feinstein Institute for Medical Research, NY; 4) Univ of California, San Francisco.

Studies of rheumatoid arthritis (RA) have consistently shown strong associations for both *HLA-DRB1* and *PTPN22*; however, the discovery of additional disease loci and complex genetic interactions is critical to further our understanding of disease pathogenesis. To identify novel gene x gene interactions in RA, we developed a two-stage analytical approach that incorporates the Random Forests (RF) algorithm and a series of association tests to uncover novel epistatic interactions. We utilized this methodology to reveal interactions with variation in the *PTPN22* locus that confer risk for RA. Multipoint identity-by-descent probabilities were determined using 292 affected RA sib-pairs for 379 microsatellite markers (MSMs) across the genome, and then used to identify predictors of *PTPN22* 1858T carrier concordance with RF. RF is a prediction technique based on a collection of classification trees grown on bootstrap samples of data using a random subset of predictors to define the best split at each node. Analyses produce a single measure of importance for each predictor. The six most important MSMs were then evaluated in 677 anti-CCP positive RA cases and 750 controls. SNPs within 5Mb of each MSM were tested first in a case-only analysis (12,367 SNPs) resulting in 21 SNPs ($p<0.001$) for follow-up which were tested for effect modification of *PTPN22* in RA cases and controls using Breslow-Day test for homogeneity. Finally, 19 SNPs ($p<0.05$) were tested for epistatic interactions using logistic models. Significant interactions ($p<0.05$) were investigated in stratified analyses. In *PTPN22* 1858T positive individuals, the presence of specific SNP alleles within four loci: *CDH13* (OR=3.82), *PRKCH* (OR=1.77), *WWOX* (OR=2.27) and *SLC35F4* (OR=1.88) significantly increased risk for RA. Our results underscore the important role of complex interactions in susceptibility to RA and the need for comprehensive analytical strategies to define them.

Severe glutathione synthetase deficiency: Long-term manifestations in two adult brothers. *K. O'Brien[1], J. Sloan[2], A. Gropman[1], E. Baker[3], T. Pierson[4], K. Fischbeck[4], I. Macdonald[5], W. Gahl[1], C. Venditti[2]* 1) MGB/NHGRI/NIH; 2) GDRB/NHGRI/NIH; 3) DRD/CC/NIH; 4) NINDS/NIH; 5) NEI/NIH.

Glutathione synthetase deficiency (GSSD) is a rare disease due to deficiency of glutathione synthetase (GSS), resulting in glutathione depletion, 5-oxoprolinuria, hemolytic anemia, and metabolic acidosis. Clinical manifestations in two adult brothers are described and compared to other patients reported with severe GSSD in order to better understand the natural history of this disorder. Two Caucasian siblings with GSSD have been followed at the NIH for more than 30 years. The older sibling was identified at 22 months of age based on typical laboratory findings; his younger brother was diagnosed as a neonate and has been treated with vitamins C and E, and alkali replacement since 2 days of life. The brothers have missense and frameshift mutations in the GSS gene. Patient 1, age 33 y, has experienced significant deterioration over the past 6 years. Previously, he was ambulating independently and working full time. Now, he is wheelchair bound, with worsening spasticity, imbalance, and dysarthria, and cannot function independently. Patient 2, age 30 y, also experienced a decline. He has developed psychosis, including paranoia, hallucinations, delusions, and motor symptoms. He ambulates with a walker and resides in an assisted living facility. A 3.0 T brain MRI/MRS revealed diffuse white and gray matter atrophy in both the cerebrum and cerebellum, ventricular enlargement, gray matter heterotopia, and a malformed cerebellum. NAA was low in 3 locations studied by MRS. These men are among the oldest known patients with severe GSSD. Over the past 6 years, both have experienced neurological decline with differing manifestations despite stable biochemical parameters. The malformed cerebellum and heterotopia seen in patient #2 are similar to findings reported in others, albeit rarely, and suggest that CNS malformations can be a feature of the disorder. The differing neurological manifestations of these two siblings also indicate that modifier genes may influence the phenotype of GSSD and highlight the importance of oxidant homeostasis in the CNS.

Modulation of recombinant protein secretion in different cell types by varying signal peptides. *A. A. Li, M. A. Potter* Department of Pathology and Molecular Medicine, McMaster University, Hamilton, Ontario, Canada.

Genetically engineered cells which express and secret therapeutic proteins can be implanted into recipients after enclosure in alginate-L-lysine-alginate (APA) capsules which provide an immune isolation of the cells. This approach has been used for gene therapy of several genetic conditions including lysosomal storage diseases, haemophilia and cancers. Efficient secretion of therapeutic protein is a prerequisite of APA microcapsule technology. In this study, we investigated whether different signal peptides and protein transduction domains would have an impact on the secretion of expressed proteins in different types of cells: mouse myoblasts (C2C12), canine epithelia cells (MDCK) and human kidney epithelia cells (293) which are widely used in microencapsulation technology. Signal peptide of human melanotransferrin p97, human factor VII, BM40 or an 11 amino acid protein transduction domain TAT has been reported to substantially enhance the secretion of some proteins which otherwise accumulated in endoplasmic reticulum. We cloned each signal peptide in-frame with a marker gene (luciferase) in pC3B vector derived from pcDNA3.1 and transfected C2C12, MDCK and 293 cells. Luciferase activities were measured in culture medium and cell lysates. Our results showed that signal peptide from p97 had the highest secretion of luciferase in 293 cells, then MDCK cells and the least in C2C12 cells. The same pattern was observed in BM40 targeted secretion, but the secretion efficiency was about half of p97 signal peptide. Although signal peptide of FVII has shown efficient secretion of other proteins, there was no improvement in secretion of luciferase in the three transfected cell lines used here. TAT showed a strong ability in retaining the protein intracellularly. Even when p97 signal peptide was fused upstream of TAT, no protein was secreted, indicating the strong retention signal of TAT exceeded the ability of p97 signal peptide to export the protein. In conclusion, it is difficult to predict a prior effect of signal peptides and protein transduction domains on protein secretion in different cell typeseach must be tested empirically.

Variation in *ABCA12* is associated with blood pressure and partially accounts for the BP linkage on 2q in the Old Order Amish. *Y. Wang[1], P. F. McArdle[1], X. Shi[1], C. M. Damcott[1], Y. C. Chang[1], B. D. Mitchell[1], A. R. Shuldiner[1,2], N. I. Steinle[1]* 1) Dept Medicine, Univ Maryland, Baltimore, MD; 2) Geriatrics Research and Education Clinical Center, Veterans Administration Hospital Medical Center, Baltimore, MD.

We previously detected strong evidence for linkage of a QTL influencing variation in blood pressure (BP) to a region on chromosome 2q in the Old Order Amish (DBP LOD=3.36; p=0.00004). Peak evidence for linkage occurred between chr2:181,883,021 and 220,376,982 (dbSNP build 126). Linkage of BP to this same region has also been reported in the Framingham Heart Study. To fine map this region, we genotyped 3,052 tag SNPs in 846 Amish subjects for association testing. These SNPs covered 58.5% of the common variation (MAF0.05, r20.8) in this region in Caucasians based on the most recent HapMap data. A total of 2,831 SNPs passed QC and were analyzed. The most strongly associated SNPs (p<0.0005) were in *SLC4A3* (rs684428, synonymous), an anion exchanger, and *ABCA12* (rs10498030, synonymous), an ATP-binding cassette. The coverage of common variations in *SLC4A3* and *ABCA12* were 66.7% and 60.4% respectively. We repeated the original linkage analysis by adding the *ABCA12* SNP genotype as a covariate and found that the magnitude of the lod score was reduced, with this SNP accounting for 24% of the original DBP linkage. None of other top DBP associated SNPs (0.0005p<0.005), including the *SLC4A3* SNP, resulted in a reduction of linkage as great as the *ABCA12* SNP, suggesting that rs10498030 partially explained the DBP linkage to 2q in the Amish. We conclude that more than one gene in this region may be responsible for the original linkage signal and that further studies of common variation as well as rare variants and copy number variants in *ABCA12* and across the region are warranted to identify the causative variants.

Small-molecule screening as a novel strategy for developing therapeutics for Gaucher disease. *O. Motabar[1], D. Urban[1], O. Goker-Alpan[1], W. Zheng[2], J. Inglese[2], C. Austin[2], E. Sidransky[1], E. Goldin[1]* 1) Section on Molecular Neurogenetics, Medical Genetics Branch, NHGRI, NIH, Bethesda, MD; 2) Chemical Genomics Center, NHGRI, NIH, Bethesda, MD.

Gaucher disease is caused by mutations in the gene encoding the lysosomal enzyme glucocerebrosidase (GBA). Most GBA mutations result in a defective protein, impaired in stability, intracellular transport efficiency or activity. The identification of small molecules with therapeutic potential that are amenable to chemical optimization may provide simple, more effective and less expensive therapy for patients with Gaucher disease Small molecules may act either by activating the enzyme or by serving as pharmacological chaperones. We initially identified new leads for treatment of Gaucher disease by screening chemical libraries assembled by the NIH Chemical Genomics Center using wildtype glucocerebrosidase (GC). A quantitative high-throughput screening assay was used to screen a collection of 62,000 compounds, identifying three different structural classes that inhibited GC at nanomolar concentrations. Representatives of these compounds were found to be effective in cell culture models. It was decided to also screen mutant GC, using preparations from patient tissue samples. In these tissue samples, the pH optimum was approximately 5.0 for both wild-type and mutant tissue samples, while the Vmax was lower for the mutant enzyme than for the wild-type. An N370S homozygote with sufficient activity was selected for the compound screen. This study will enable us to pinpoint the functional units in the protein and to design potent drugs for patients with Gaucher disease.

The functional consequences of genetic variation on *tyrosine hydroxylase* (*TH*) expression. *L. R. Warner*[1,2], *C. C. Babbitt*[1], *A. E. Primus*[1], *G. A. Wray*[1,3] 1) Institute for Genome Sciences & Policy, Duke University, Durham, NC; 2) Developmental Biology Program, Duke University, Durham, NC; 3) Department of Biology, Duke University, Durham, NC.

Tyrosine hydroxylase (TH) is the rate-limiting enzyme in catecholamine synthesis and variations in the promoter region of *TH* have been implicated in disease states. However, the impact of variation in *cis* between humans and other primates, as well as within humans, is not well understood. Our study investigated naturally occurring haplotypes in *Homo sapiens*, *Pan troglodytes*, *Gorilla gorilla*, and *Macaca mulatta*. We used a dual luciferase reporter system in two neuroblastoma cell lines to assay differences in expression level based on sequence differences within the 2 kb region upstream of the *TH* translation start site. We observed several significant differences in expression between haplotypes from primate species, but the most striking difference was observed between human haplotypes. We identified two SNPs in humans that regulate the expression of *TH* in a specific cellular environment. Transcription factor binding algorithms predict an allele specific change in transcription factor binding at the two SNPs sites. Using a site-directed mutagenesis approach on the two SNPs of interest we have obtained informative results regarding the genetic basis of TH functionality. In sum, *TH* expression within humans is modulated by *cis*-regulatory sequence variants, highlighting the functional importance of natural genetic variation in *cis*.

A high density mapping study identifies association to the SLAM locus in SLE. *P. Goyette[1], D. S. Cunninghame Graham[2], T. J. Vyse[2], P. R. Fortin[3], A. Sharpe[4], C. Terhorst[5], J. Wither[6], Canios. GenES Investigators[4], J. D. Rioux[1]* 1) University of Montreal and Montreal Heart Inst, Montreal, Canada; 2) Molecular Genetics and Rheumatology, Imperial College Faculty of Medicine, London, UK; 3) University of Toronto Lupus Clinic, Centre for Prognosis Studies in the Rheumatic Diseases, Toronto Western Hospital, University Health Network; Department of Medicine, University of Toronto, Canada; 4) Harvard Medical School, Pathology, NRB-837, Boston, USA; 5) Division of Immunology, BIDMC, Harvard Institute of Medicine, Boston, USA; 6) Arthritis Centre of Excellence; Division of Genetics and Development, Toronto Western Hospital Research Institute, University Health Network; Departments of Medicine and Immunology, University of Toronto, Canada.

Systemic lupus erythematosus (SLE) is a complex disease trait of unknown aetiology. Genome-wide linkage studies in SLE have identified several linkage regions, including 1q23, which contains the signalling lymphocyte activation molecule (SLAM) locus. In mice there is a syntenic linkage region, Sle1. The SLAM genes are functionally related cell-surface receptors, which regulate signal transduction in immune cells. A recent association study in UK/Canadian SLE families identified the SLAMF7-LY9 region as contributing to SLE susceptibility. The strongest association, rs509479, resulted in a M602V amino acid change in LY9 which has potential functional consequences. We undertook a high density association study including 272 SNPs capturing the genetic variation at the SLAM locus as well as missense polymorphisms in SLAM genes. The analysis was performed in 671 UK cases, and initial results reveal two areas of SLE associations at the SLAM locus: the SLAMF6-VANGL2 region and the previously reported LY9-SLAMF7 region. We are currently performing an independent replication study using a cohort of 270 Canadian trios, collected through the GenES/CaNIOS Consortium. If the association to this region is confirmed these results, in combination with the discoveries from genome-wide association studies (GWAS), will provide additional evidence that genetic variation in genes with key functions in adaptive immunity are involved in the pathogenesis of SLE.

Genetic and functional studies of mutations in PPP1R3F, a novel PP1 regulatory subunit, in X-linked mental retardation. *J. Wang[1], F. Abidi[2], A. Hackett[3], A. Adamczyk[1], C. Skinner[2], K. Holden[2], F. L. Raymond[4], P. Tarpey[5], M. Stratton[5], J. Gecz[6], R. Stevenson[2], C. Schwartz[2], T. Wang[1]* 1) Johns Hopkins University, Baltimore, MD; 2) Greenwood Genetic Center, Greenwood, SC; 3) University of Newcastle, NSW, AU; 4) University of Cambridge, UK; 5) Wellcome Trust Sanger Institute, UK; 6) University of Adelaide, Adelaide, AU.

Mental retardation (MR) is a common cause of severe handicap in children and young adults affecting 2-3% of the general population. Genetic causes for mental retardation are heterogeneous involving many cellular pathways and processes. Phosphorylation of key synaptic proteins is essential for the induction and maintenance of LTP and LTD, two cellular models of learning and memory. Protein phosphatase 1 (PP1) plays an important role in the regulation of protein phosphorylation in neurons. PP1 activity and specificity are determined by numerous interacting regulatory subunits. Using a combination of cDNA microarray and candidate gene approach, we identified PPP1R3F, a putative PP1 regulatory subunit, as a candidate gene for X-linked mental retardation (XLMR). We conducted a mutation survey in a cohort of male probands (~400) and identified four synonymous and six nonsynonymous coding variants (nsCV). Four nsCV involve highly conserved residues in predicted functional domains, segregate with MR phenotype, and are absent in 500 normal males. On Northern blot, PPP1R3F showed abundant transcripts in brain, fetal brain, testis, and skeletal muscle. Using coimmunoprecipitation (CoIP) and a yeast two-hybrid (Y2H) assay, we detected strong interaction of PPP1R3F with both PP1-CA and PP1-CC subunits, confirming that PPP1R3F is a novel PP1-interacting protein. On immunoblot, these mutations generate stable protein in transfected HEK293 cells. In preliminary CoIP studies, at least two mutants showed a reduced interaction with PP1-CA, which are being replicated using Y2H assay. Results suggest that PPP1R3F mutants have a causative role in cognitive impairment in patients with XLMR. Further studies of these mutants will help to understand the role of PPP1R3F in the modulation of PP1 activity and in cognitive development and function in humans.

***CSMD1* Genetic Variation and Human Dextrality: Whole Genome Association Results.** *T. Lencz[1,2,3], P. DeRosse[1,2], K. E. Burdick[1,2,3], J. M. Kane[1,2,3], A. K. Malhotra[1,2,3]* 1) Center for Translational Psychiatry, Feinstein Institute for Medical Research, Manhasset, NY; 2) Dept Psychiatry Research, Zucker Hillside Hosp, Glen Oaks, NY; 3) Dept. of Psychiatry & Behavioral Sciences, Albert Einstein College of Medicine, Bronx, NY.

Background - Left-handedness (or, more broadly, nondextrality) is a moderately heritable trait that has been associated with increased risk for several psychiatric, neurologic, and learning disorders. As such, nondextrality may represent an easily assessed phenotype reflecting neurodevelopmental processes related to cerebral asymmetries. To date, however, only a single candidate gene study for handedness has been reported, and a small number of linkage studies have yielded equivocal results. The present genomewide association study provides a hypothesis-free method to examine the genetic underpinnings of nondextrality.

Methods - Handedness was assessed using the Edinburgh Handedness Inventory in 396 Caucasian individuals as part of a case-control study of schizophrenia at the Zucker Hillside Hospital. Sixty-four subjects (16%) were nondextral (laterality score <0.70). Genotyping was performed using the Affymetrix 500K microarray; QC yielded ~325K high-quality, common (MAF0.05) autosomal SNPs. The significance threshold for chi-square tests was defined a priori as p<4.2*10-7.

Results - One SNP (rs4639542) was significantly associated with nondextrality under additive, recessive, and allelic models. This SNP lies within an intron in *CSMD1*, the third largest gene in the human genome. The minor (C) allele demonstrated an odds ratio of 2.86 (95% CI=1.92-4.27) for nondextrality; CC homozygosity yielded 7-fold increase in risk. Results were consistent irrespective of subject sex or diagnosis.

Conclusions - *CSMD1* is a complement regulatory gene on chromosome 8p23.2 and is highly expressed in the brain. As such, this gene is a plausible neurodevelopmental candidate, and may also provide explanation of the previously reported association of left-handedness and autoimmune disorders.

MDB: A multicentric web-based database to study human malformative phenome. *JC. Fournet[1,2], F. Arbabzadeh[1,2], P. Sawicki[1], D. Bouron-Dal Soglio[1], H. Kabbara[2], E. Lemyre[3], LL. Oligny[1]* 1) Department of Pathology, Sainte-Justine Hospital, Montreal, QC, Canada; 2) Research Center, Sainte-Justine Hospital, Montreal, QC, Canada; 3) Genetics, Department of Pediatrics, Sainte-Justine Hospital, Montreal, QC, Canada.

Human developmental anomalies are still poorly described by a open free-access web-based database. Our purpose was to construct a user-friendly database dedicated to human developmental anomalies in order to describe statistically malformative combinations (sequences, associations, complexes, syndromes, diseases). We constructed a MySQL database describing the different entities involved in malformative pathology (called MDB for Malformation DataBase): developmental anomalies, malformative sequences, malformative associations, malformative syndromes, malformative diseases, chromosomal anomalies, malformation genes. We built an ontology of human developmental anomalies according to the OBO consortium (Open Biomedical Ontologies at http://www.obo.org). In a pilot series, we entered 200 cases of foetus with malformation from the autopsy series of the Department of Pathology of Sainte-Justine Hospital (Montreal, QC, Canada) observed from 2001 to 2007. The MDB Malformation DataBase website can be accessed at URL http://www.malformations.org. This pilot series permitted us to validate the structures and functions of our database. It permits to easily generate statistical data as frequencies of developmental anomalies by organs, by syndromes or by diseases. For malformative combinations, the database will permit us to study and quantify aggregations of developmental anomalies in malformative combinations and to give them a graphic representation (data visualization). It will permit to classify observed foetus and malformative syndromes according to a phenotypic clustering graph. We now want to associated other centers in foetopathology to perform a statistical study of 1000 foetus with developmental anomalies (For you want to participate to this study, you can contact us at join@malformations.org).

Cognitive endophenotypes to genetically dissect Alzheimer disease. *S. Barral[1], J. H. Lee[1], R. Cheng[1], V. Santana[1], J. Williamson[1], R. Lantigua[1], M. Medrano[2], I. Jimenez[3], E. Rogaeva[4], Y. Stern[1], P. George-Hyslop[4], R. Mayeux[1]* 1) Taub Institute & Sergievsky Ctr,Columbia Univ,New York, NY 10032; 2) Univ Tecnol de Santiago, Santiago, DR; 3) Department of Internal Medicine, Univ of Puerto, San Juan, PR; 4) Ctr for Research in Neurodegenerative Diseases, Univ of Toronto, Toronto, Canada.

Alzheimer Disease (AD) diagnosis relies on the assessment of memory performance and other cognitive functions. We and others showed that memory, a strong AD predictor, is highly heritable, and therefore may be used as endophenotype to genetically dissect AD. We propose that there might be distinct pathways involving distinct genetic factors: 1) those associated with early memory decline that eventually lead to AD; 2) those associated with memory decline, but do not lead to AD; 3) those associated with AD without significant cognitive decline. We examined 3 regions: two of them, 3q28 and 5p15, were previously reported in our genome-scans as strong associated with AD. The third region, 2q24, represents a more modest AD signal. We studied verbal memory (total recall, delayed recall and recognition) in 1068 subjects from 210 Hispanic families. We performed 2-point variance component analysis adjusting for age, sex and education. We also created composite cognitive index (CCI) -- a weighted combination of total recall, delayed recognition, and education which predicts individuals AD risk -- to further investigate 2q24. LODs of 3.1 and 1.9 were observed for AD at 3q28 and 5p15, respectively. A modest LOD of 0.53 was found in 2q24. For total recall, delayed recall and recognition, LODs were 2.2, 1.7 and 2.4 for 3q28; 1.3, 0.9 and 7.9 for 5p15. LODs in 2q24 region were <1. However, we found a LOD of 1.45 for CCI, which increases to 2.43 when restricted to unaffecteds. The results suggest that paths may differ at these 3 loci. Results in 5p15 and 2q24 imply that genetic factors may influence memory and CCI directly but not AD, since the results became stronger after removing AD subjects. 3q28 might harbor AD genes that are not associated with significant memory decline. Here we have shown that memory and related traits can be used to dissect AD.

Precise identification of a supernumerary chromosome by high density microarray. *I. GADI[1], M. HORNER[2], D. DAY-SALVATORE[2], J. TEPPERBERG[1], V. JASWANEY[1], P. PAPENHAUSEN[1]* 1) CYTOGENETICS, LABORATORY CORPORATION OF AMERICA, RTP, NC; 2) INSTITUTE FOR GENETIC MEDICIANE, ST. PETERS'S UNIVERSITY HOSPITAL, 254 EASTON AVENUE,NJ 08903-0591.

An eight year old adopted female with global developmental delay, mental retardation, dysmorphia and hyperactivity was referred for whole chromosome SNP microarray analysis. A previous cytogenetic and FISH analysis had revealed a supernumerary chromosome derived from chromosome 9 detected through whole chromosome paint probes. However, additional FISH for the pericentromeric region of chromosome 9 was negative. A high resolution whole genome microarray (Affymetrix Inc.) based analysis of 1.8 million SNP/copy number probes confirmed a gain of the chromosome 9 short arm, although the three copy dosage did not extend into the centromeric region of 9p. The SNP array also revealed a significant gain of the chromosome 13 proximal long arm. A 36.2 Mb gain of 9p21.3-->9pter included the linear base pair position extending from 263651 to 20,502,190 Mb was evident from the SNP array results. A chromosome 13 gain of 3.273 Mb included the linear base pair position 17,943,628-->21,216,442 of 13q11 to 13q12.11. These results indicate that the reported supernumerary chromosome is composed of 9p and a smaller, apparent centromeric portion of proximal 13q. The chromosome microarray and FISH results are consistent with a supernumerary derivative chromosome 13 [der(13)t(9;13)(p11;q12)] which has most likely resulted from a 3:1 meiotic segregation of a balanced parental translocation involving chromosomes 9 and 13. Unfortunately biological parents of this adopted child were not available for additional studies. High-resolution SNP microarray can be utilized for rapid and precise resolution of marker chromosome composition that can accurately define genomic imbalance necessary for genotype-phenotype correlation.

Polymorphisms of iron-related genes and childhood leukemia risk. *C. Davis, T. Do, M. T. Dorak* Genomic Immunoepidemiology, HUMIGEN LLC, the Institute for Genetic Immunology, 2439 Kuser Road, Hamilton, NJ 08690.

Hemochromatosis gene (HFE) variants that increase body iron levels also increase the risk for childhood acute lymphoblastic leukemia (ALL). We extended the original HFE association study in 114 cases with childhood ALL and 414 newborn controls from Wales (UK) to 20 SNPs in iron-related genes (IRG): HFE2 (hemojuvelin), HFE4 (ferroportin), TF (transferrin), TFRC (transferrin receptor), HAMP (hepcidin), homolog genes HEPH (hephaestin) and CP (ceruloplasmin), HMOX1 (heme oxygenase1) and BMP2 (bone morphogenetic protein 2) as well as 13 SNPs in HFE and its flanking regions. Univariate analysis showed a strong protective association with HFE flanking region SNP rs807212 (OR=0.32; P<0.0001 in males) which accounted for the original male-specific C282Y association. There were other protective associations in males (TF rs1049296 and rs4481157), in females (HMOX1 rs2071748) and with no sex-specificity (HFE2 rs4970862) with marginal statistical significance. While univariate associations were weak, their additive effect (P for gene-dosage effect <0.0001 in boys) and interactions were strong. HAMP and HEPH/CP interacted with TFRC with sex effect, and HFE2 interacted with BMP2 and HFE4. BMP2 and HFE2 are both involved in an iron sensing pathway and statistical interactions observed corresponded to biologic interactions among these molecules and HFE. The combination of HFE2 rs4970862, HFE4 rs2304704 and BMP2 rs235756 conferred risk (OR=3.7; P=0.02) in girls, but the same association was non-significant in boys (OR=2.4, P=0.15). We previously reported the interactions between HFE and TFRC increasing birth weight in males which would confer slight protection from genotoxic effects of excess iron. Overall, iron-related genes modify childhood leukemia risk possibly due to their effect on placental iron transport. Our interpretation is that greater iron transport to the fetus would increase the risk for leukemia in girls, whereas boys would be less affected owing to their ability to increase cell proliferation rate to reduce excess iron. Boys would therefore only be at risk at extreme iron excess which would also lead to excessive birth weight.

Acute Intermittent Porphyria: Development of AAV2/8 Gene Therapy. *L. Gan*[*1], *M. Yasuda*[*1], *D. F. Bishop*[1], *R. J. Ziegler*[2], *S. H. Cheng*[2], *R. J. Desnick*[1] 1) Genetics and Genomic Sciences, Mount Sinai Sch Medicine, New York, NY; 2) Genzyme Corporation, Framingham, MA.

Acute Intermittent Porphyria (AIP) is an autosomal dominant inborn error of heme biosynthesis due to the half-normal activity of hydroxymethylbilane synthase (HMBS). The most common hepatic porphyria is manifested by life-threatening, acute neurological attacks precipitated by various drugs, fasting, alcohol, and hormonal changes. Current therapy for acute attacks involves intravenous infusion of hematin, which can be venotoxic and is short-lived. Thus, development of a preventative therapy is desirable, especially for patients with frequent attacks. To design an adeno-associated viral (AAV) vector with optimal transgene expression, four unique combinations of liver-specific enhancers and promoters were evaluated for hepatic transgene expression by transient transfection of HepG2 cells and hydrodynamic delivery in mice. The 1-microglobulin enhancer/1-antityrpsin promoter combination achieved the highest transgene expression *in vitro* and *in vivo* (*Yasuda et al., J Gene Med 9:806, 2007*). These regulatory elements were used to generate a recombinant AAV8-based serotype vector encoding murine HMBS (rAAV2/8-HMBS), and AIP mice (*Lindberg et al., Nat Genet 12:195, 1996*) were used to assess the efficacy of AAV-mediated therapy. Intraperitoneal injection of rAAV2/8-HMBS showed a time- and dose-dependent increase of HMBS, most marked in the liver. Notably, administration of 7.6×10^{11} particles - the highest dosage tested - achieved a 1.5-2 fold increase of hepatic HMBS over wildtype that was sustained for 8 weeks. Treated mice were resistant to the characteristic accumulation of porphyrin precursors when induced by serial phenobarbitol injections, indicating that the expressed enzyme was functional *in vivo*. These studies demonstrated that AAV-mediated gene therapy can correct the biochemical defect in AIP mice, and thus, may be a promising preventative therapy for patients with recurrent acute attacks. Further studies are currently underway to evaluate the safety and long-term efficacy of AAV-mediated gene therapy for AIP. (*equal authors).

Common variants in *STK39* are associated with blood pressure levels. *Y. Chang[1], Y. Wang[1], J. O'Connell[1], P. McArdle[1], J. Wade[2], S. Dorff[1], S. Shah[3], X. Shi[1], L. Pan[4], E. Rampersaud[1], H. Shen[1], J. Kim[5], A. Subramanya[2], N. Steinle[1], P. Welling[2], C. Ober[4], A. Weder[6], A. Chakravarti[7], B. Mitchell[1], A. Shuldiner[1]* 1) Division of Endocrinology, Diabetes and Nutrition, University of Maryland School of Medicine, Baltimore, MD; 2) Department of Physiology, University of Maryland School of Medicine, Baltimore, MD; 3) Section of Cardiology, Department of Medicine, University of Chicago, Chicago, IL; 4) Department of Human Genetics, University of Chicago, Chicago, IL; 5) Georgetown University School of Medicine, Washington, DC; 6) Department of Internal Medicine, University of Michigan School of Medicine, Ann Arbor, MI; 7) Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD.

Hypertension places a major burden on individual and public health, but the genetic basis of this complex disorder is poorly understood. We conducted a genome-wide association study of systolic and diastolic blood pressure (SBP/DBP) in Amish subjects and found strong association signals with common variants in a serine/threonine kinase, *STK39*. We confirmed this association in an independent Amish and 4 non-Amish Caucasian samples including the Diabetes Genetics Initiative, Framingham Heart Study, GenNet, and Hutterites (combined N=7,125, $P<10^{-6}$). The higher BP-associated alleles have frequencies > 0.09 and were associated with increases of 3.3 and 1.3 mmHg in SBP/DBP, respectively, in Amish subjects and with smaller but consistent effects across the non-Amish studies. *In vitro*, STK39 is known to interact with WNK kinases and cation-chloride cotransporters, mutations in which cause monogenic forms of BP dysregulation. We demonstrate that *in vivo*, *STK39* is expressed in the distal nephron, where it may interact with these proteins. While none of the associated SNPs alter protein structure, we identified and experimentally confirmed a conserved intronic element with allele-specific *in vitro* transcription activity as a functional candidate for this association. Hence variants in *STK39* may influence BP by increasing STK39 expression and altering renal Na^+ excretion, thus unifying rare and common BP-regulating alleles in the same physiologic pathway.

Factors affecting the risk of early menopause in carriers of *FMR1* premutations. *A. Murray[1], C. E. Bennett[1], P. A. Jacobs[2], J. N. Macpherson[2]* 1) Peninsula Medical School, University of Exeter, Exeter, United Kingdom, EX1 2LU; 2) Wessex Regional Genetics, Salisbury District Hospital, Salisbury, Wilts, UK SP2 8BJ.

The *FMR1* gene contains a polymorphic CGG repeat in exon 1 which has been associated with three phenotypes; Fragile X Syndrome (FXS), Fragile X Tremor and Ataxia Syndrome (FXTAS) and Premature Ovarian Failure (POF). FXS is caused by inactivation of *FMR1*, usually by methylation of the gene as a result of expansion of the CGG to over 200 copies. FXTAS and POF are both associated with unmethylated, premutation sized repeats and are thought to be caused by an RNA gain-of-function mechanism. We have investigated the role of the CGG repeat in ovarian failure in over 500 women ascertained because of ovarian failure and nearly 400 women from fragile X families. Contrary to published data and in a larger study population, we do not find an association with large normal or intermediate sized repeats (35-55 CGG) and POF, in 351 cases compared to 1564 controls (p=0.55). In premutation carriers POF was more likely in women who had a repeat of between 80 and 100 CGGs, no AGG interruptions, and a FRAXE GCC repeat allele below the population mean.

Endometrial cancer and somatic G>T *KRAS* transversion in patients with constitutional *MUTYH* biallelic mutations. *M. Genuardi[1], B. Ciambotti[1], P. Bet[2], B. Gatteschi[3], V. Gismondi[2], B. Toschi[1], L. Varesco[2], R. Tricarico[1]* 1) Dept. Clinical Pathophysiology, Medical Genetics, Univ Florence, Florence, Italy; 2) National Cancer Institute, Hereditary Tumour Unit, Genoa, Italy; 3) National Cancer Institute, Pathology Unit, Genoa, Italy.

MUTYH-associated polyposis (MAP) is an autosomal recessive condition predisposing to colorectal cancer, caused by inherited defects in the base excision repair gene *MUTYH*. Colorectal tumours from biallelic *MUTYH* mutation carriers display an excess of somatic G>T mutations in the *APC* and *KRAS* genes due to defective BER function. To date, few extracolonic manifestations have been observed in MAP patients, and the clinical spectrum of this condition is not yet fully established. Recently, one patient with a diagnosis of endometrial cancer and biallelic *MUTYH* germline mutations has been described. However, it is not yet clear if *MUTYH* mutations increase the risk of endometrial tumours. We here report on two unrelated MAP patients with biallelic *MUTYH* germline mutations who developed endometrial carcinoma. The endometrial tumours were evaluated for *PTEN*, *PIK3CA*, *KRAS*, *BRAF* and *CTNNB1* mutations. A G>T transversion at codon 12 of the *KRAS* gene was observed in one tumour. A single 1bp frameshift deletion of *PTEN* was observed in the same sample. Overall, these findings provide additional evidence for the inclusion of endometrial carcinoma among the phenotypic manifestations of MAP. In addition, they indicate that MAP should be considered as a differential diagnosis with Lynch syndrome for patients with colorectal and endometrial tumours.

Participants' attitudes towards Biobank Japan. *K. Muto[1], C. Chiungfang[1], S. Suzuki[2], I. Kobayashi[2], Y. Nakamura[1]*
1) Dept Public Policy, IMS, University of Tokyo, Tokyo, Japan; 2) Shonan Kamakura Hospital, Kanagawa, Japan.

[Purpose and Method] Biobank Japan is the largest sample bank donated by 200,000 Japanese patients with various diseases. Paticipants are asked to donate serums and clinical information once a year since 2003 other than DNA. To evaluate our quality of consent process and exlore participants' motivation to keep participating, we've been conducting a questionnaire survey since 2007.[Subjects] Patients of one hospital in the suburbs of Tokyo were asked to respond our questionnaires at their regular visits for donation. [Results] 459 participants responded (M:F=1:0.73, RR=70.). 80% of them responded that they would keep participating and 65% didn't feel any burden for donation. However, 39% had already fogotten what they were informed when they gave consent to participate.[Discussions] The initial consent process was not enough to keep participants remember the purpose of the research project and why they were asked to donate. We need to make efforts to seek better communication between participants and scientists.

A novel approach for obtaining confidence sets of SNPs regulating quantitative phenotypes. *C. Papachristou*[1], *S. Lin*[2] 1) Math/Phys/Stat, University of the Sciences in Philadelphia, Philadelphia, PA; 2) Statistics, The Ohio State University, Columbus, OH.

As the genetic maps become highly dense, the desire to sufficiently localize putative disease loci has become an achievable goal. This has prompted an increased interest in methods for constructing confidence intervals for the location of trait contributing genes. Such intervals are very important since, by reducing the number of candidate genes, they can help us design cost-effective and time-efficient follow-up studies. We introduce a new approach that can be used in whole genome scans to obtain a confidence set of quantitative trait loci (QTLs) contributing at least a predetermined percentage *h* to the overall genetic variation of a quantitative phenotype. The method is developed in the framework of the generalized linear mixed models and can accommodate families of arbitrary size and structure. Specifically, we assume the availability of a dense SNP map consisting of *S* markers and for each SNP *s, s=1,..., S*, we test the following hypotheses: $H_{0s}: {}^2_{G_s} h^2_{G_T}$ *vs.* $H_{0s}: {}^2_{G_s} h^2_{G_T}$, where $^2_{G_s}$ is the genetic variance attributed to SNP s, $^2_{G_T}$ is the total genetic variance of the quantitative phenotype, and *h* is a number between zero and one chosen in advance. Obviously, the set of markers for which the above null hypothesis is not rejected at level constitutes an *(1-)×100%* confidence set of loci contributing at least *h×100%* percent of the total genetic variance of the phenotype. Implementation of the method needs knowledge of the total genetic variance of the trait which can be readily estimated, usually with good accuracy, from the data themselves. Our simulation results under various genetic models and different levels of relevant factors, such as family structure and size, confirm that our method provides confidence sets that maintain the nominal coverage. Furthermore, with just 250 nuclear families with two offspring, our method was able to place the causative SNP in a set of at most 3 SNPs, even when the QTL contributed as little as 10% to the total phenotypic genetic variance, while at the same time it held the false positive rate below acceptable levels.

Abnormal karyotype acute myeloid leukemia (AML) occurring after normal karyotype AML represents therapy-induced secondary disease: an array CGH analysis. *S. Sait[1], G. Deeb[2], D. Gaile[3], L. Sheperd[3], S. Lui[3], P. Starostik[2], L. A. Ford[4], M. Wetzler[4], J. Conroy[5], N. Nowak[5], E. S. Wang[4]* 1) Clinical Cytogenetics Lab; 2) Department of Pathology and Laboratory Medicine; 3) Department of Biostatistics; 4) Leukemia Service, Department of Medicine; 5) Microarray Facility, Department of Cancer Prevention and Control, Roswell Park Cancer Institute, Buffalo,NY.

Although karyotype instability (defined as the acquisition of further aberrations in addition to those present at diagnosis) has been reported to occur in up 10-30% AML patients with normal primary diagnostic karyotype, the etiology and clinical significance of these abnormalities is uncertain. While possible that cryptic chromosomal aberrations were present at diagnosis but not detected by standard cytogenetic methods due to dilutional effects; alternatively, these abnormal karyotype AMLs may represent secondary leukemias resulting from intensive chemotherapy regimens. We examined 26 de novo normal karyotype (NK) AML patients at diagnosis found to have abnormal karyotype (AK) AML at relapse/disease persistence. Ten cases were FAB-M2, seven were FAB-M1, six FAB-M4, two FAB-M5a, and one FAB-M0. Median age was 64 years; gender was evenly split. Chromosomal abnormalities included unbalanced and balanced structural rearrangements, complex karyotypes, and numerical changes. Rearrangements did not include commonly reported abnormalities, except for one patient with trisomy 8. Aberrations commonly associated with secondary AML i.e. -5/5q-, -7/7q- and 11q23 abnormalities were observed in two patients with -7/7q-. High-resolution array-based comparative genomic hybridization (aCGH) revealed no copy number aberrations in 11/12 NK/AK diagnostic patient samples examined but confirmed the presence of multiple abnormalities in 2/2 NK/AK relapsed patient samples, consistent with conventional cytogenetic results. Our data suggest that the majority of cases of karyotypically abnormal AML following a diagnosis of normal karyotype AML represent secondary leukemic disease resulting from therapy-induced DNA damage rather than clonal dilutional effects of the original AML clone.

Risk for Autism is Increased With Advanced Parental Age and Education in an Iranian Population Sample. *R. Sasanfar[1,2,4], S. Haddad[1], A. Tolouei[3], M. Ghadami[4], S. L. Santangelo[1,2,5]* 1) Psychiatric and Neurodevelopmental Genetics Unit, Center for Human Genetic Research, Massachusetts General Hosp, Boston, MA, USA; 2) Dept. Psychiatry, Harvard Medical School, Boston, MA, USA; 3) Diagnosis and Prevention, Special Education Organization, Tehran, Iran; 4) Ministry of Education, Special Education Organization, Tehran, Iran; 5) Dept. Epidemiology, Harvard School of Public Health, Boston, MA, USA.

The etiology of idiopathic autism is still unclear and despite its strong heritability, few susceptibility genes have been identified. Evidence from several epidemiological studies has implicated parental age as a risk factor for autism. Recently implemented population screening for autism in Iran allowed for a large-scale epidemiological study to investigate the effect of parental age on risk for autism spectrum disorders (ASD) in an Iranian population sample consisting of 179 ASD cases and 549,354 cohort comparison children, aged 5-12. ASD diagnoses were made by DSM-IV criteria and standard diagnostic instruments. Analyses used logistic regression, adjusting for maternal age, parental education, birth order, consanguinity, and sex. We found a ~2-fold increased risk for ASD with increasing paternal age over 36 years. Compared with fathers in the age range 26-30, the odds ratio for fathers aged 36-40 was 1.99 (95% CI: 1.24, 3.21) while the OR for fathers > 40 years was 1.83 (95% CI: 1.02, 3.30). Maternal age > 26 years showed a more striking 4-fold increased risk for autism in a matched case-control analysis (OR: 4.1, 95% CI: 1.35, 12.93). Risk for ASD was also increased for a combination of higher parental age and education. Compared to young, uneducated parents, older parents with a college degree or graduate school had a ~5-fold risk of having a child with ASD (95% CI: 2.29, 11.15), with a clear dose-response relationship. Results from this study may be explained by an increased likelihood of DNA damage and/or improperly imprinted genes being transmitted by older parents. However, we cannot rule out the possibility that the apparent increase in risk with higher education may be due to greater health-care seeking behaviors in more educated parents.

A 15 Year Review of A Pediatric Down Syndrome (DS) Clinic. *M. Carlin, J. Spahis* UT Southwestern Medical School/Children's Medical Center, Dallas, Texas.

In 1993, the Center initiated a weekly clinic for pediatric patients with DS from birth to 18 yrs.. There have been 1923 new patients seen during 2523 visits through March, 2008; 815 patients were <12 mos. at first visit. A multidisciplinary team of professionals from genetics, nursing, PT, speech, social work and a parent from the local support group provide care as recommended in *Preventative Health Care for Children with Genetic Conditions*, Wilson, GN & Cooley, WC: 2nd ed., Cambridge University Press, 2006. Data have been collected from families and health records and entered into a Microsoft Access 2002 database. Ethnic background is: Caucasian 44%, Hispanic 44%, African 8%, Asian 3%, Other 1%. Karyotypes indicate 96% with non-disjunction trisomy 21, 3% translocations and 1% mosaicism. Common findings noted during the first year include: neonatal jaundice in 328(40%), thrombocytopenia 17(2%), congenital heart defect(s) 440(54%), GE reflux 276(34%), GI anomalies 118(14%) and congenital hypothyroidism 19(2%). Initial c-spine screens were obtained on 682 at 3 yrs. of age. Of those, 65(10%) showed instability. Other important findings across all ages are: recurrent respiratory infections 711(37%), PE tubes 684(36%), strabismus 385(20%), constipation 251(13%) and leukemia 9(<1%). Periodic screening detected acquired hypothyroidism in 82 patients.

Clinic impact is reflected in the improved preventative management for patients with DS in the community. Fewer children required thyroid and c-spine screening in 2007 (35% thyroid; 16% c-spine) than in 1993 (43% thyroid; 25% c-spine). A similar drop occurred in referrals for early intervention (10% in 1993; 4% in 2007). Interestingly, of the 35 patients with c-spine instability who were re-studied 1 yr. later, 22(63%) had normal films. Additionally, parents have expressed appreciation for advice and support from other parents during clinic visits.

Common variants in MTNR1B (melatonin receptor 1B) influence fasting glucose and type 2 diabetes risk. *C. Langenberg[1], I. Prokopenko[2,3], J. Florez[4,5,6], R. Saxena[4,7], G. Thorleifsson[8], N. Soranzo[9,10] for MAGIC* 1) MRC Epidemiology Unit, Cambridge, UK; 2) Oxford Centre for Diabetes, Endocrinology and Metabolism, Oxford, UK; 3) Wellcome Trust Centre for Human Genetics, Oxford, UK; 4) Broad Institute of Harvard and MIT, Cambridge, MA; 5) Department of Medicine, Harvard Medical School, Boston, MA; 6) Center for Human Genetic Research and Diabetes Unit, Massachusetts General Hospital, Boston, MA; 7) Center for Human Genetic Research, Massachusetts General Hospital, Boston, MA; 8) deCODE genetics, Reykjavík, Iceland; 9) Wellcome Trust Sanger Institute, Hinxton, UK; 10) Twin Research and Genetic Epidemiology Department, King's College London, London, UK.

Fasting glucose (FG) levels usually are tightly regulated within a narrow physiologic range, and disruption of normal glucose homeostasis and elevation of FG are hallmarks of insulin resistance and type 2 diabetes (T2D). To identify novel loci that impact on FG we established MAGIC (Meta-Analyses of Glucose and Insulin-related traits Consortium), a collaborative effort of 10 individual studies represented by four consortia: ENGAGE (deCODE, NFBC66, NTR/NESDA, Rotterdam Study), GEM (CoLaus, TwinsUK), DFS (DGI, FUSION, SardiNIA) and the Framingham Heart Study. We initially exchanged the identities of 10-20 SNPs most prominently associated with FG from GWA scans in each study. All four groups (n=6,828-12,390) independently identified strong evidence for association at MTNR1B (melatonin receptor 1B), confirmed by meta-analysis of the 1Mb region flanking the gene (n=36,610; 381 SNPs). The risk allele of the SNP most strongly related to higher FG levels (per allele increase in FG 0.07 mmol/L [95% CI: 0.06-0.08]; $P=1.1x10^{-41}$) also showed evidence of association with HOMA-B ($P=1.0 \times 10^{-15}$), as well as an increased risk of T2D (per allele odds ratio = 1.12 [95% CI: 1.07-1.18]; $P=5.2 \times 10^{-6}$) in a meta-analysis of six case-control studies totalling 7,625 cases and 45,419 controls. In addition, we confirm the previously reported associations of variants at the G6PC2 ($P=7.1 \times 10^{-49}$) and GCK ($P=4.8 \times 10^{-20}$) loci and FG levels. We conclude that MTNR1B is a novel candidate gene involved in FG regulation and T2D risk.

Investigation of rare and common variants in resequencing data from 313 patients with dilated cardiomyopathy.

R. E. Hershberger[1], E. Rampersaud[2], K. Hamilton[2], S. Khuri[3], D. Li[1], J. Cowan[1], A. Morales[1], E. R. Martin[2] 1) Cardiovascular Division, University of Miami; 2) Miami Institute for Human Genomics, University of Miami; 3) Center for Computational Science, University of Miami.

Dilated cardiomyopathy (DCM) is a myocardial disease characterized by left ventricular enlargement and systolic dysfunction (ejection fraction less than 50%), and is a common cause of heart failure. Although mutations, primarily nonsynonymous missense or short indels, found in >20 autosomal and two X-linked genes have been shown to cause familial DCM (FDC), this variation explains only ~25-30% of genetic cause. Therefore, in an effort to identify additional causative variations, we undertook a comprehensive examination of resequencing data provided by the National Heart Lung and Blood Institute (NHLBI) from six known FDC genes (MYH7, TNNT2, SCN5A, CSRP3, LBD3, and TCAP) in 313 carefully phenotyped DCM patients and 253 controls. In total, 524 variation sites were examined, 252 of which fell in the 5UTR or 3UTR or resulted in nonsynonymous (NS) or synonymous nucleotide changes. Of highest interest were 36 NS changes that were absent in all controls. Since these alterations accounted for only a small fraction of the total genetic variability we then performed association analyses of all sites using a Fishers exact allelic test. After accounting for deviations from Hardy Weinberg Equilibrium and linkage disequilibrium, we identified 12 independent sites across all genes that were associated ($P<0.05$) with DCM. Removal of the 36 individuals with NS changes did not alter the significance of these associations, suggesting the presence of additional variation not captured by these rare variants. Furthermore, we observed a larger number of multiple hits across different genes in DCM cases compared to controls. This analysis suggests that common susceptibility variants alone or in combination may contribute to additional risk. This investigation highlights the importance of using a comprehensive approach to analyze extensive resequencing data sets, even in diseases that have previously been categorized as Mendelian.

The Effect of Background Selection on Patterns of Human Genetic Variation. *R. Hernandez, M. Przeworski* Human Genetics, University of Chicago, Chicago, IL.

Recent studies have demonstrated that the human genome is rife with deleterious mutations, arising in both coding as well as conserved non-coding regions. However, the possible effects of such a concert of deleterious mutations on flanking neutral variation has received little attention in human population genetics, despite background selection (BGS) having been described 15 years ago. To examine the possible effects of BGS on the human genome, we used detailed forward simulations with demographic, selective, and recombination parameters inferred from human data. We first investigated the effect of deleterious non-synonymous mutations on patterns of synonymous variation within the same gene. We found that while inference of selection at non-synonymous sites remains largely unbiased, the frequency distribution of neutral synonymous mutations can be significantly impacted by BGS in a non-stationary population. We extended our simulations to incorporate a genomic distribution of functional elements subject to deleterious mutations to characterize the spatial scale that BGS can affect. Observed patterns of variation near functional elements from large-scale human resequencing projects were then contrasted to the expected pattern of BGS to quantify the evidence for BGS as a driving force in the evolution of the human genome.

Genetic associations in childhood leukemia and interactions with sex. *M. T. Dorak, E. Ucisik-Akkaya, C. Davis, B. Morrison, T. Do* Genomic Immunoepidemiology, HUMIGEN LLC, Hamilton, NJ, 2439 Kuser Road, Hamilton, NJ 08690.

To gain further insight into previously reported associations with HLA genes, we examined the non-HLA genes of the HLA complex and a number of other candidate genes involved in iron homeostasis, immune surveillance and genome surveillance using a genic approach. The sample consisted of 114 cases with childhood acute lymphoblastic leukemia (ALL) and 414 newborn controls from Wales (UK). Most HLA complex associations were sex-specific (HSPA1B in males); SKIV2L, HLA-C and -DRA in females (similar to their associations in autoimmune diseases). Retinoic acid receptor gene (RXRB) showed an association which appeared to account for the HLA-DPB1 association previously reported. The DRB4 association originally found in the same sample remained the strongest risk marker for males. The most striking finding was a heterozygote advantage observed in males at three extended HLA complex loci (HFE rs807212, BAT3 rs2077102 and DRB ancestral lineages). A strong additive effect of these markers was noted ($P < 0.001$) and showed remarkable protection in newborn boys heterozygous at all three markers (OR = 0.05, 95% CI = 0.01 to 0.43; $P = 0.007$). SNPs at IRF4, TP53, several iron-related genes (TF, HFE2, HMOX1) and an NKG2D haplotype known to correlate with lower natural cytotoxic activity also showed associations and biologically plausible interactions. The African HapMap sample was monomorphic for the protective HSPA1B allele G. Associations shown to be independent resulted in a genome-wide additive susceptibility model which was highly significant ($P < 0.0001$). Some markers associated with risk in males or protection in females also showed decreased frequency in newborn males (HLA-DRB lineages, NKG2D and HMOX1). This finding provided a genetic basis for the link between male-specific prenatal selection and childhood ALL susceptibility suspected from the miscarriage history and leukemia risk connection (stronger in males). Further studies in the HLA complex which encodes more than 200 expressed non-HLA genes are justified given the genetic associations, heterozygote advantage and possible involvement in prenatal selection, all with sex effect, found in the present study.

Application of the Bayesian Regression with Singular Value Decomposition Method in Large-Scale Candidate Gene Association Studies for Insulin Resistance. *S. Kwon[1], X. Guo[1], M. o. Goodarzi[1], Y.-D. I. Chen[1], B. Fang[1], S. Cheng[2], V. Brophy[2], J. Li[2], L. Steiner[2], A. Xiang[3], K. D. Taylor[1], T. A. Buchanan[3], L. J. Raffel[1], J. I. Rotter[1]* 1) Medical Genetics Inst, Cedars-Sinai Medical Ctr, Los Angeles, CA; 2) Roche Molecular Systems, Pleasanton, CA; 3) USC, Los Angeles, CA.

Genetic association studies have been widely used to identify genes involved in a disease pathophysiology. With the increasing number of reported positive associations, it is an important and challenging task to sort through the signals and identify the genes that confer the highest risk for disease development. We applied the Bayesian Regression with Singular Value Decomposition (BRSVD) method for large-scale genetic association studies when sample size (n) is much smaller than the number of markers (p). Massive dimension reduction from p to n can be achieved by applying SVD to the design matrix. The posterior densities of all parameters in the model were driven with conjugate priors. Model fitting can be accomplished via Markov chain Monte Carlo with Gibbs sampler, which can be constructed based on posterior densities. Test procedures based on permutation and generalized likelihood ratio are incorporated to select significant genes. We applied our BRSVD method to candidate gene data generated in a Hispanic American (HA) family cohort ascertained through probands with hypertension. A total of 227 SNPs from 103 candidate genes have been genotyped. The BRSVD method was used to assess all the genes simultaneously, using 103 offspring selected from the family cohort (one from each family with complete phenotype and genotype data) with the goal of identifying genes important for insulin resistance (IR), as measured directly by euglycemic clamp. In addition to age and BMI, 5 genes (CD14, GPRK2L, MMP7, CAPN10, and CD36) were found to contribute to IR significantly ($p<0.05$), with CAPN10 ranked at the top. This is a provocative finding given that CAPN10 was the first gene for T2DM identified by positional cloning and was initially discovered in a HA cohort. We propose that the BRSVD method will improve gene identification in large scale genotyping studies.

Novel Genetic Variants of TCF7L2 identified by direct sequencing in Mexican Mestizos and Amerindians. *L. del Bosque-Plata, A. C. Hernandez-Mondragon, G. Jimenez-Sanchez* National Institute of Genomic Medicine, Mexico.

Intronic variants of the transcription factor 7-like 2 gene (TCF7L2) has been associated with type 2 diabetes (T2D). This association has been consistently replicated among diverse populations with different genetic backgrounds including Mexican population. These variants do not explain the original linkage signal, raising the possibility that other variants in the same region may predispose to T2D. Based on these observations showed the importance of direct sequencing this gene, especially if the replication cohort is of different ethnic group compared to the original one. Most of the Mexican population is considered Mestizo with a particular genomic ancestry that is the result of admixture between Amerindians with Spaniards and a lesser amount with Africans population. About 5% of Mexican population are Amerindian groups. We looked for new TCF7L2 variants by direct sequencing of all exons and intron-exon junctions in three Mexican Amerindian groups: 30 Zapotecan, 45 Mazatecan and 30 Mexican Mestizos. We have preliminary found four novel TCF7L2 variants in the Amerindian population. These were found in exon or intron regions: exon 17: Pro-His, Pro-Thr; intron 13: del CC, ins Pro490His, Pro502Thr, DelCC +209392, InsTCTCTC +209397. Some of these variants were localized in transcriptional binding sites. A second phase of this study is planned to look for these novel variants in Mexican population Mestizos by case-control association design. Identification of novel variants in TCF7L2 will contribuye to our knowledge of the patogénesis of T2D.

Telomere length in brain and psychiatric disorders. *D. Zhang, L. Cheng, C. Liu* Department of Psychiatry, University of Chicago, Chicago, IL.

Telomeres consist of a variable number of repeats of the sequence TTAGGG at the ends of chromosomes, involve in preventing from chromosome fusion and maintaining genome stability. Telomeres decline with cell division. Telomere shortening has been recently observed in leucocytes from psychological stress and psychiatric disorders such as schizophrenia (SZ), bipolar disorders (BD) and major depression (MD). We aim to test whether telomere length is altered in brain of psychiatric patients including MD, BD and SZ. Mean telomere length (mTL) varies greatly in humans with the same age. Linkage studies have suggested that several regions contribute to mTL. We also carried out a genome-wide mapping of mTL as a quantitative trait. We measured mTL in 155 Caucasian cerebellum samples from Stanley Medical Research Institute (SMRI) by real time PCR. These samples include 46 SZ, 46 BP, 15 MD and 48 healthy controls. No difference of mTL was observed comparing SZ (or BD or MD or all cases) and controls. Affymetrix Genome-Wide Human SNP Array 5.0 was employed for genotyping. 240576 markers with high quality were included in subsequent mapping analysis. SNP rs605863 showed the most significance with p value of 8.87E-06. No significance withstood multiple test corrections.

Expression profiling study on human monocytes identified novel MicroRNAs:miR-126, miR-151 and miR-152 for osteoporosis. *L. G. Sun[1], X. D. Chen[1], R. R. Recker[1], H. W. Deng[2], P. Xiao[1]* 1) Osteoporosis Research Center, Creighton University Medical Center, Omaha, NE,68131; 2) Department of Orthopedic Surgery and Basic Medical Sciences, University of Missouri-Kansas City, Kansas City, MO, USA, 64108.

MicroRNAs (miRNAs) are short noncoding RNA molecules that regulate gene expression by targeting mRNAs and causing mRNA cleavage or translation blockage. Recently, miRNAs have been implicated important in the etiology of various diseases. Osteoporosis is characterized by low bone mineral density (BMD) mainly resulting from imbalanced osteoclastogenesis and osteoblastogenesis. Circulating monocytes actively participate in osteoclastogenesis by serving as osteoclast precursors and producing factors important for the development and function of osteoclasts. There may be important miRNA-regulating mechanisms in circulating monocytes for the etiology of osteoporosis. In this study, we recruited 20 unrelated postmenopausal Caucasian females aged 57-68, 10 with high BMD (spine or hip Z-score > 0.84) and 10 with low BMD (spine or hip Z-score < -0.84). Total RNA (including miRNAs) from circulating monocytes for each subject was extracted and reverse-transcribed. miRNA profiling for each sample was performed using the ABI TaqMan Low Density Array, including 365 miRNA probes. miRNA expression levels were evaluated by comparative Ct method (Ct) using RNU48 as the endogenous control. Differentially expressed miRNAs were determined by t-test. We identified three down-regulated miRNAs, miR-126 (p=0.023), miR-151 (p=0.015) and miR-152 (p=0.035), in the low BMD group vs. the high BMD group. Another study showed that miR-126 affected hematopoietic stem cell (HSC) differentiation by regulating the expression of Hoxa9 gene, which is important in myeloid and T-cell differentiation as well as HSC function, suggesting that miR-126 may function in osteoclast differentiation. However, no previous study has identified the function of miR-151 and miR-152. This is the first in vivo miRNA expression study on human monocytes for osteoporosis. Our results suggest that miR-126, miR-151, and miR-152 may be involved in osteoclastogenesis and thus the etiology of osteoporosis.

Genomewide association study of schizophrenia in European ancestry and African American samples. *J. Shi[1], D. F. Levinson[1], A. R. Sanders[2], J. Duan[2], F. Dudbridge[3], I. Pe'er[4], P. Holmans[5], S. L. Bray[3], S. Gusev[4], B. J. Mowry[6], R. Freedman[7], A. Olincey[7], F. Amin[8], C. R. Cloninger[9], J. M. Silverman[10], N. G. Buccola[11], W. F. Byerley[12], D. W. Black[13], P. V. Gejman[2]* 1) Dept Psychiatry/Behavior Sci, Stanford Univ, Stanford, CA; 2) Dept Psychiatry, Evanston Northwestern Healthcare Research Inst, Evanston, IL; 3) MRC Biostatistics Unit, Cambridge, UK; 4) Dept Computer Sci, Columbia Univ, New York, NY; 5) Dept Psychol Med, Cardiff Univ, Cardiff, UK; 6) Queensland Ctr Ment Hlth Research, Univ Queensland, Brisbane, Australia; 7) Dept Psychiatry, Univ Colorado, Denver, CO; 8) Dept Psychiatry, Emory Univ, Atlanta, GA; 9) Dept Psychiatry, Washington Univ, St. Louis, MO; 10) Dept Psychiatry, Mt. Sinai SOM, New York, NY; 11) School of Nursing, LSUHSC, New Orleans, LA; 12) Dept Psychiatry, UCSF, San Francisco, CA; 13) Dept Psychiatry, Univ Iowa, Iowa City, IW.

We are reporting a genomewide association study of schizophrenia (SZ) on ~ 5,700 European-ancestry (EA) and 2,400 African-American (AA) cases and controls from the Molecular Genetics of Schizophrenia collaboration. Cases were recruited by 10 centers under a common protocol. Samples were genotyped with the Affymetrix 6.0 array, with extensive QC filtering. To control for population substructure, principal component scores were computed, outliers excluded, and ten scores included as covariates in logistic regression analyses (trend tests). Preliminary analyses include half of the EA dataset (1351/1378 cases/controls post-QC) and 95% of the AA dataset (1199/954 cases/controls), genotyped by the GAIN program. Post-QC genomic control lambdas were ~1.03 (AA) and ~1.07 (EA, EA+AA). The strongest signals were in the FAM69A/RPL5/EVI5 cluster (p=10-6, EA) and in MAD1L1 ($p=2x10^{-6}$, EA; and $7x10^{-8}$, EA+AA), C10orf59 ($4x10^{-6}$, AA) and ERBB4 ($4.5x10^{-6}$, AA). Evidence for association to FAM69A/RPL5/EVI5 has been reported in multiple sclerosis. ERBB4 is the receptor for the product of NRG1, a SZ candidate gene which also received support here ($p=3x10^{-5}$, AA). We will present analyses that include an additional ~ 2800 EA and 130 AA samples, imputation of all HapMap SNPs, and analyses of copy number variants.

A flexible and accurate genotype imputation method for the next generation of genome-wide association studies.

B. N. Howie[1], P. Donnelly[1,2], J. Marchini[1] 1) Dept Statistics, Univ Oxford, Oxford, United Kingdom; 2) Wellcome Trust Centre for Human Genetics, Univ Oxford, Oxford, United Kingdom.

The past couple years have seen a flurry of novel disease associations arising from genome-wide scans, and these successes have been facilitated by the rise of genotype imputation methods. To date, most genome-wide imputation analyses have used HapMap as a reference. This strategy has proven fruitful, but imputation based on a (relatively) small number of haplotypes is inherently limited in the sorts of patterns it can capture; these limitations disproportionately affect rare SNPs, which are an important source of power in imputation analyses. In the near future, publicly available datasets (such as the Wellcome Trust Case Control Consortium controls and samples from the 1,000 Genomes Project) will serve as rich reference panels that can be used to increase power in association studies. These new opportunities also present new challenges for imputation, however, such as the computational and modeling difficulties posed by much larger samples of possibly unphased and incomplete genotypes. Building on the established method IMPUTE, we describe a new method (IMPUTE++) for genotype imputation whose main innovations are to model such data efficiently and to seamlessly integrate new reference panels with existing ones. We illustrate our method by performing a cross-validation experiment using the WTCCC 1958 Birth Cohort samples with genotypes from the Affymetrix 500K and Illumina 550K SNP chips, where 1000 of the samples are used as an expanded reference panel to impute genotypes in the remaining 500. We show that applying IMPUTE++ to this expanded reference panel yields substantially more accurate results than leading methods can attain based on HapMap alone: our approach often cuts error rates by a factor of 1.5-2.0, across rare (MAF < 5%) and common SNPs alike. We also apply simpler methods based on multi-marker tags to the expanded reference panel dataset and obtain error rates more than 2X ours. Our approach is quite general, and provides a robust foundation for analyzing new datasets (e.g., the 1,000 Genomes Project) and conducting meta-analyses to boost power even further.

Macrocephalic sperm head syndrome: about four familial North African cases. *T. Rebai[1], R. Frikha[1], R. Louati[1], L. Lamine[2], N. Abdelmoula[1]* 1) Histology, Medical University , Sfax, Tunisia; 2) Private Sector of gynecology, Sfax, Tunisia.

Men presenting with teratozoospermia associated with large heads and multiple-flagella spermatozoa are now considered to have the macrocephalic sperm head syndrome, also known as meiotic division deficiency witch accounts for <1% of male infertility. Analysis of the sperm chromosomal content using FISH demonstrated a relationship between the aneuploidy and polyploidy rate and the rate of large-headed multiple-tailed spermatozoa. Recently, a common region of homozygosity harboring the aurora kinase C gene (AURKC) with a recurrent single nucleotide deletion in the AURKC coding sequence has been reported in macrocephalic sperm head syndrome. In the present study, we report on genetic analysis in four infertile North African men having macrocephalic sperm head syndrome (two brothers from a first Libyan family (F1) and two other brothers from a second Libyan family (F2)). In the first family, the patients were born from first degree cousins but in the second, the brothers were born from non consanguineous parents. The F1 brothers (45 and 42 years old men) had 18 and 2 years history of infertility, normal blood lymphocyte karyotype and 100% of large heads and multiple-flagella spermatozoa in semen investigations. They had familial history of a sister with mental retardation children and a brother who failed to conceive because multiple pregnancy losses. The F2 brothers (43 and 38 years old men) had 12 and 5 years history of infertility, normal blood lymphocyte karyotype, severe OAT and large heads and multiple-flagella spermatozoa in semen. For the first brother, two ICSI attempts had been done without results: failure of fecundation for the first attempt and failure of pregnancy in the second. Molecular investigation mainly by sequencing the AURKC gene is performed in our cases. Results will be discussed in the light of literature.

***TRIM32* in limb girdle muscular dystrophy type 2H/sarcotubular myopathy.** *P. L. Miller, H. Ding, Y. Heng, A. Kania, X. Wu, A. Funk, C. Hirst, S. Krawitz, M. DelBigio, K. Wrogemann* University of Manitoba, Winnipeg, Manitoba, Canada.

Limb Girdle Muscular Dystrophy Type 2H is a relatively mild form of muscular dystrophy that occurs commonly in the Hutterite population. A homozygous point mutation (D487N) in TRIM32 was found in all patients. A congenital non progressive sarcotubular myopathy (STM) was also described in Hutterites and in two German patients with the same mutation, indicating they represent just one disease. Re-evaluating the pathology of past biopsies from 12/14 LGMD2H patients shows varying degrees of vacuolation in scattered fibres, in agreement with the genetic findings. A different mutation (P130S) leads to one form of Bardet-Biedl Syndrome (BBS). A knockout mouse model shows signs of muscular dystrophy and vacuolation characteristic of LGMD2H/STM and thus confirms that the correct gene has been identified. Additional mutations in LGMD patients have recently been found by others, and all map to one of the six NHL domains. Elevated levels of wt Trim32 in skin carcinogenesis and the observation that mutated TRIM32 leads to muscular dystrophy suggested that TRIM32 exerts anti-apoptotic activity (Albor et al., J. Biol. Chem. 281: 25850-66, 2006). Semi-transformed human myoblasts show greater susceptibility to staurosporine induced apoptosis. This should not necessarily indicate that increased apoptosis underlies the pathogenesis of LGMD2H/STM. It could just be a result of the disease process. The gene is highly expressed in regenerating skeletal muscle. When myoblasts are cultured under conditions of differentiation those from LGMD2H patients show reduced levels of myosin heavy chain and caveolin, indicative of a possible impairment in the differentiation process. TRIM32 is an E-3 ubiquitin ligase. Monoclonal Abs have been developed and detect TRIM32 in most tissues with highest expression in brain and testis, while tissues from knock-out mice show no signal. Skeletal muscle biopsies from LGMD2H patients show reduced levels of TRIM32, which is also seen in myoblasts and fibroblasts from 2H patients. The precise roles of TRIM32 mutations in the pathogenesis of LGMD2H remain to be elucidated.

A Delphi-based Consensus Clinical Practice Protocol for Very Long Chain Acyl-CoA Dehydrogenase (VLCAD)Deficiency. *G. L. Arnold[1], D. Matern[2], J. VanHove[3], D. Freedenberg[4], N. Longo[5], B. Burton[6], C. Garganta[7], C. Ficicioglu[8], S. Cederaum[9], C. Harding[10], R. Boles[11], A. Feigenbaum[12], P. Chakraborty[13], A. Strauss[14]* 1) U Roch SOMD, Rochester, NY; 2) Mayo Clinic COM, Rochester, MN; 3) U Colo HSC, Denver, CO; 4) Vanderbilt U SOM, Nashville TN; 5) U Utah SOM, Salt Lake, UT; 6) Northwestern U Feinberg SOM, Chicago, IL; 7) Tufts NEMC, Boston, MA; 8) U Penn SOM, Philadelphia, PA; 9) UCLA SOM, Los Angeles, CA; 10) Oregon HSU, Portland, OR; 11) USC SOM, Los Angeles, CA; 12) Hosp Sick Child, Toronto, ON; 13) Child Hosp E Ontario, Ottawa, ON; 14) U Cinn SOM, Cincinnati, OH.

VLCAD deficiency is diagnosed by newborn screening with relatively high frequency (1:31,500 births). Although most newborns appear well, it is important to determine which infants are at risk for the severe cardiomyopathy phenotype vs. the milder phenotypes of fasting intolerance or later onset myopathy. Evidence based guidelines for diagnosis and management of screen positive infants are lacking. After a review and grading of the literature (per the Oxford Centre for Evidence Based Guidelines), 14 metabolic experts developed consensus based clinical practice guidelines for VLCAD screen positive infants using the traditional Delphi consensus method.

Recommendations were considered for the initial evaluation of the screen positive infant, diagnostic testing, and management. Plasma acylcarnitine (AC) analysis was recommended as a follow-up test; because of false-negative AC studies a second diagostic method (DNA, enzyme asay or fatty acid oxidation probe) was recommended to verify normal AC. At present there are insufficient data to predict severity of phenotype from biochemical assays alone, however preliminary data suggest genotype or residual enzyme activity may help guide management. Age and phenotype-specific recommendation for dietary treatment (long chain fat restriction and medium chain triglyceride supplementation) were considered, as were recommendations for management of fasting during intercurrent illnesses. Carnitine remains controversial, although a slender consensus recommended supplementation if plasma carnitine is deficient.

Prediction of Antidepressant Drug Response in Geriatric Depression. *J. Sarginson, L. Lazzeroni, H. S. Ryan, A. F. Schatzberg, G. M. Murphy* Psychiatry and Behavioral Sciences, Stanford University School of Medicine, Stanford, CA.

Background: Polymorphisms that can help predict medication treatment outcomes in geriatric major depression would be of great clinical value. Because the clinical efficacy of antidepressant agents depends on their concentrations in the brain, we focused on the MDR1 gene, which encodes the blood brain barrier transporter p-glycoprotein. P-glycoprotein affects the concentrations of some antidepressants such as paroxetine, but not others such as mirtazapine. Methods: We performed a multicenter pharmacogenetic study of 246 cognitively intact patients 65 years of age and older with major depression who were treated with either paroxetine or mirtazapine. Medications were administered in a double blind design over an 8-week acute phase with a 16-week extension phase. Mood, cognition, and medication side effects were quantified throughout the study. All individuals were genotyped for 15 SNPs spread across the MDR1 gene region. Statistical analyses were performed using mixed model regression on Hamilton Depression Scale 21 item (HamD-21) scores, and Kaplan-Meier survival analysis, using time to a HamD-21 score of 10 or less as a criterion for remission from depression. Results: Of the 15 MDR1 variants, only rs2032583 was a significant predictor of time to remission. Two other SNPs, rs2032582 and rs1045642, which are the most frequently genotyped MDR1 variants and have been associated with response to cardiac and chemotherapeutic medications, had no effect on antidepressant outcomes for either drug. Conclusion: These results indicate that MDR1 variation may affect treatment outcomes for geriatric patients given p-glycoprotein substrates such as paroxetine. This finding partially supports a previous finding in younger depressed patients (Uhr et al., Neuron: 57:203, 2008). We did not confirm a second association between rs2032582 and response to paroxetine in a Japanese cohort of depressed patients. Nevertheless, our results support further testing of MDR1 as a pharmacogenetic predictor in patients treated with p-glycoprotein substrate antidepressants. Support: NARSAD, Organon Inc., Department of Veterans Affairs, The Nancy Pritzker Network, and the NIMH.

Werner mesomelia with Hirschsprung disease in an affected father and son. *S. Ramanathan[1], Y. Ibrahim[2], C. Gurnett[3], R. D. Clark[1]* 1) Peds Genetics, Loma Linda Univ Hlth Care, Loma Linda, CA; 2) NICU Dept, Loma Linda Univ Hlth Care, Loma Linda, CA; 3) Dept Neurology, Univ of Washington School of Med, St Louis, MO.

We report a father and son with Werner mesomelic dysplasia and Hirschsprung disease. Fetal anomalies in the son noted on ultrasound at 20 weeks gestation were preaxial polydactyly of hands and a single bone in the lower legs. At 24 weeks, a double outlet right ventricle (DORV) was detected. The baby was born at 37 weeks by C-section to a 35-year-old G1 mother. He had bilateral preaxial synpolydactyly of hands and feet with 7 digits on each extremity, bilateral triphalangeal thumbs, short lower legs, talipes and a grade II systolic murmur. X-rays showed synpolydactyly with 7 digits of hands and feet, triphalangeal thumbs, radial deviation of hands, absent tibiae and talipes. Other anomalies were Hirschsprung disease and DORV. The babys father had below the knee amputations for absent tibiae. He also had asymmetric thumbs, preaxial polydactyly of feet, cardiac murmur and Hirschsprung disease. Werner mesomelia is a rare disorder characterized by absent or hypoplastic tibiae and preaxial polysyndactyly of the extremities. Only 2 cases of Werner mesomelia with Hirschsprung have been reported. To our knowledge, this is the first report of a familial case of Werner mesomelia with Hirschsprung disease. ZRS is a highly conserved domain 1 Mb upstream of Sonic Hedgehog (*SHH*) and regulates *SHH*. Mutations in ZRS have been reported in isolated preaxial polydactyly (Lettice *et al*., 2002), preaxial polydactyly and triphalangeal thumbs (Gurnett *et al*., 2007), and in Werner mesomelic dysplasia without Hirschsprung disease (Wieczoreg *et al*., ESHG abstract, 2008). ZRS studies in our family are in progress. Further studies are needed to determine if the severity of the Werner mesomelia phenotype and the presence of Hirschsprung disease are associated with specific mutations in ZRS. As the Hedgehog signaling pathway is critical in developmental patterning during embryogenesis, ZRS may play a role in the etiology of Hirschsprung disease and congenital heart disease even in the absence of recognized limb anomalies.

Addition of CYP450 2C9*8 and CYP450 4F2 to a dosing algorithm for Warfarin dosing. *P. Hujsak[1], P. Wang[2], A. Smith[3], A. Wu[3]* 1) AutoGenomics, Inc, Carlsbad; 2) Cornell University,Chemistry Laboratory, Methodist Hospital, Houston; 3) Clinical Chemistry Laboratory, San Francisco General Hospital, San Francisco.

The anticoagulant warfarin with its patient dependent narrow individual therapeutic range makes it difficult for a physician to prescribe a maintenance dose without repeated follow up INR testing. Patients sensitive to warfarin are at high risk of serious bleeding events. It has been shown that genetic variations in the CYP450 2C9 (*2, *3, *5, *6, *8, *11, *12 and *14) enzyme in the liver affect the rate of metabolism and clearance of warfarin. Warfarin inhibits the target vitamin K epoxide reductase complex-1 (VKORC1) enzyme activity.VKORC1 genetic variations at the 3673 (G:A), and the 7566 (C>T) positions implicated in warfarin dosing.The CYP4504F2 (V433M) enzyme has also been linked to Warfarin dosing. We retrospectively genotyped 71 patients on warfarin on an INFINITI automated platform, and compared the genotypes against the actual dose used to maintain a stable INR. The group is also highly diverse (36% Caucasian, 33% African American, 17% Asian, 10 Hispanic, 2% other). We used this data to create an algorithm based on age, gender, height, weight, inhibitors, ethnicity, and smoking. The algorithm we developed uses results of *2, *3, *8 for 2C9 and 3673 and 7566 VKORC1 and CYP4F2. The algorithm is: Log dose=1.1931-0.0008972(Age)-0.1389(asian)-0.09422(black)-0.06092(*2)-0.1846(*3)-0.1233(*8)+0.01019(4F2)+0.1102(gender)-0.09022(Hispanic)-0.001234(height)-0.01173(inhibitor)+0.05277(other)-0.04342(smoking)-0.1516(3673)-0.04646(7566)+0.0009239(weight) (2,*3 and *8 are still number of alleles, while VKORC13673=3 for AA, 2 for AG and 1 for GG,VKORC1 7566=3 for TT, 2 for TC and 1 for CC, 4F2=3 for TT, 2 for TC and 1 for CC) The regression coefficient improved slightly to r2=0.59with the additions of CYP 2C9 *8 and CYP 4F2 relative to to 2C9*2 and *3 and VKORC1 3673, 7566. There were 38.4% heterozygous samples of 4F2. The CYP2C9 *8 SNP was present 5.6% of this sample set. It is more prevalent in the African American population than *2. With continuing testing of a mixed population, more CYP SNPs will be uncovered that affect the dosing algorithm.

***IL18* locus is associated with susceptibility to esophageal disease in an Irish Population.** *M. Babar[1], G. Turner[1], A. W. Ryan[1], P. J. Murphy[1], A. E. Hughes[2], C. C. Patterson[2], L. A. Anderson[2], R. G. Watson[2], B. T. Johnston[2], J. McGuigan[2], H. Comber[2], L. J. Murray[2], J. V. Reynolds[1], R. McManus[1], FINBAR CONSORTIUM* 1) Trinity College Dublin & St. James' Hospital, Dublin, Dublin, Ireland; 2) Department of Medical Genetics, Queen's University Belfast, Royal Group of Hospitals, Belfast, Ireland.

Esophageal Adenocarcinoma (EAC) is multifactorial and the genetic background may be a crucial etiologic factor. Interleukin-18 is a multifunctional cytokine implicated in anti-tumour immunity. Encoded on chromosome 11q22.2_q22.3, variation in the *IL18* gene promoter has been associated with esophageal squamous cell carcinoma in the Chinese population. These promoter polymorphisms have been reported to alter *IL18* expression and have been linked to nasopharyngeal and ovarian cancers. **Methods** We analysed two *IL18* promoter polymorphisms, -137 G/C and -607 C/A, (rs187238 and rs1946518) in *IL18* gene on 11q22.2_q22.3. Each SNP was genotyped in 1708 individuals (EAC, n=192, Barretts, n=201, Reflux esophagitis, n=205, Controls,n=1110). We investigated the relationship between these SNPs and their haplotypes with the risk of esophageal disease. **Results** The data from all loci were in Hardy-Weinberg Equilibrium in all populations. The genotype frequencies of the *IL18* promoter polymorphism -607 C/A were significantly different between controls and Barretts (p=0.04) and marginally different between controls and EAC (p=0.07). The CC genotype was significantly associated with an increased risk of Barretts esophagus (p=0.012, OR=1.456) and slightly increased risk of EAC (p=0.06, OR=1.02). Although no significant association was observed between the disease groups at the -137 G/C locus, the -137C/-607C haplotype was associated with significantly increased risk of Barretts esophagus (p=0.032) with haplotype frequencies 0.596 in controls and 0.605 in Barretts. **Conclusion** These data shows a suggestive association of the Barretts population with the *IL18* -607 C/A promoter polymorphism. Higher *IL18* expression caused by the -607C allele might contribute to an inherited susceptibility to Barretts esophagus and possibly EAC.

Whole Genome Analysis of DNA Methylation Abnormalities in Breast Cancer. *A. H. O'Donnell[1,2], J. R. Edwards[3], C. Lee[5], H. Peckham[5], F. Haghighi[4], T. H. Bestor[1]* 1) Genetics and Development; 2) MD/PhD Program; 3) Columbia Genome Center; 4) Psychiatry, Columbia University, New York, NY; 5) Applied Biosystems, Foster City, CA, USA.

The nature of the DNA methylation abnormalities known to occur in cancer cell genomes remains controversial, in large part because of the lack of a method for analyzing the methylation patterns of the entire genome including repetitive elements in a rapid, cost-effective, unbiased manner. We have developed new methods for the fractionation of DNA according to methylation status and have employed ultra-high throughput DNA sequencing using the Applied Biosystems SOLiD platform to allow efficient whole-genome methylation profiling. A new computational pipeline to organize the flood of sequence data generated during this study has been implemented. We have assessed methylation status of nearly the entire genome in two breast cancers and adjacent normal breast tissues and the breast cancer cell line MCF-7 to create the largest methylation databases to date. We have found that the default program of DNA methylation is driven by local CpG density. However, CpG-dense promoters are shielded from methylation by histone H3K4 di- and tri-methylation marks. Young repetitive elements have higher methylation levels than predicted by CpG density, suggesting that there may be a mechanism that targets methylation of this class of sequences. From our analysis, we have detected a vast number of methylation changes between normal and tumor samples across the genome including in promoters, repetitive elements and other genomic regions, and are now examining the role these changes may play in breast cancer. Interestingly, promoter hypermethylation is not selectively present at tumor suppressor genes, but can also be seen at proto-oncogene promoters, supporting a role for DNA methylation in a hypermethylation/demethylation suicide pathway that kills cells that have lost mechanisms of growth control.

An association study of *CNR1* polymorphisms in schizophrenia with or without cannabis use. *K. Prasad, J. Wood, M. Talkowski, K. Chowdari, V. Nimgaonkar* Dept Psychiatry, Univ Pittsburgh Sch Med, Pittsburgh, PA.

Background: Cannabis use is associated with increased risk of psychosis or schizophrenia (SZ). Variations in endogenous cannabinoid system are observed in SZ independent of cannabis use. This suggests that pathology in the endocannabinoid system could predispose to both SZ and cannabis abuse. However, association of the gene encoding cannabis receptor 1 (*CNR1*) variations with SZ in the context of cannabis use has not been adequately examined. We comprehensively examined the association of *CNR1* polymorphisms with SZ with and without cannabis abuse/dependence. **Methods**: Consenting SZ patients (n=527) and healthy subjects (n=477) were administered the Diagnostic Interview for Genetic Studies (DIGS) and a consensus diagnosis was made. We selected 17 tag SNPs from HapMap based on a high correlation threshold (r^2<0.9). SNPs were genotyped using the Illumina Golden Gate platform (ABI Biosystems, Inc). We examined the association of tag SNPs with SZ, and then between those with history of cannabis abuse/dependence compared to those without. **Results**: SNPs rs806370 (p=0.048) and rs806365 (p=0.071) showed nominal associations with SZ but did not survive corrections for multiple tests. When patients with (n=121) or without (n=335) cannabis abuse/dependence were compared, rs1049353 (synonymous coding; ^{2}=9.2, p=0.024) and rs806369 (intronic; ^{2}=7.2, p=0.072) were significantly associated with SZ patients with cannabis abuse/dependence after correcting for effective number of tests (9.73). The odds ratio was 1.65 and 1.62, respectively. **Discussion**: These observations lend initial clues to the possibility that variations in *CNR1* may be associated with a subset of SZ patients who are abusing or dependent on cannabis. A small sample size did not allow us to reliably examine the onset of cannabis use relative to the onset of SZ. To our knowledge, this study utilizes one of the largest systematically characterized samples. We are, now, examining similar associations among bipolar disorder patients, and investigating the impact of these SNPs on in vivo neurobiological variables among first episode antipsychotic naïve SZ patients relative to matched healthy controls.

Y chromosome microsatellite haplotypes in the Hutterite founders. *M. Caliskan[1], I. Pichler[2], C. Platzer[2], P. P. Pramstaller[2], C. Ober[1]* 1) Human Genetics, U of Chicago, USA; 2) Institute of Genetic Medicine, European Academy, Bolzano, Italy.

The current population of >12,000 Schmiedeleut Hutterites are descendants of 38 male founders who were born between 1700 and 1830 in Europe. Only 12 of these founders, each with a unique surname, have living male descendants related through male-only lineages. DNA samples were available in our laboratory for 75 male descendants of 11 of the 12 founders, accounting for 673 independent paternal meioses. We genotyped 9 microsatellite loci, which included a mean of 6.8 (range 2-23) males per lineage to evaluate potential relationships between the founders. Fourteen different haplotypes were identified, with an average of 3.5 (range 1-8) pairwise differences between haplotypes. All descendants within each of 9 lineages had identical Y haplotypes. Descendents of two of these lineages, 2 and 10, had the same haplotype despite different surnames, suggesting possible relatedness between the founders of these two lineages. Descendants of two lineages, 6 and 11, each carried three distinct haplotypes. Within each of these lineages the haplotypes differed from the ancestral haplotype by one repeat size at two loci. Additional male descendants in lineages 6 and 11 were then genotyped for the discrepant microsatellites, confirming the presence of three Y haplotypes each in lineages 6 and 11. The one mutation arose at each of four loci: DYS388, DYS389II, DYS390, DYS393. Three mutations were gains of one repeat; it was not possible to determine if the fourth mutation was a gain or loss of one repeat. The ancestral haplotypes in these two lineages are identical at four microsatellite loci; the alleles at the other five loci differ by one repeat size. The average mutation rate at these 9 loci was 0.00066 (95% CI 0.00015-0.0013), similar to other estimates. These data suggest that the founders of lineages 2 and 10 may have been related through paternal lines and that surnames do not strictly correspond to unique Y chromosomes. Moreover, certain ancestral haplotypes (i.e., those in lineages 6 and 11) may be more prone to mutation. Supported by NIH grants HD21244 and HL085197.

A new model of Down Syndrome: human BAC transgenic for PCP4. *Y. Khvorostova[1,6], G. Barlow[1], D. L. Dickstein[2], D. Segal[2], P. R. Hof[2], L. S. Crnic[3], D. Patterson[4], T. Town[1], Z. Galdzicki[5], J. R. Korenberg[6]* 1) Cedars-Sinai Medical Ctr., Los Angeles, CA 90048; 2) Mount Sinai School of Med., New York, NY; 3) Univ. of Colorado School of Med., Denver, CO; 4) Eleanor Roosevelt Inst., Denver, CO; 5) Uniformed Services Univ. of the Health Sciences, Bethesda, MD; 6) The Brain Institute Univ. of Utah, Salt Lake City, UT.

Down syndrome (DS) or trisomy 21 is a common genetic disorder, and mental retardation based on memory and learning defects remains the hallmark of DS. These cognitive functions are dependent on hippocampus (HC) and prefrontal cortex (PFC) function, and are mediated in part by the effects of chromosome 21 genes expressed in these regions acting on neuronal structure and function. The HSA 21 gene, PCP4, encodes the protein PEP-19 and is expressed in PFC and HC and binds calmodulin. This results in diminished activity of downstream enzymes such as calmodulin kinase II, and may thereby influence learning and memory. Here, we present a new PCP4 DS mouse model. We have used a human BAC transgenesis strategy to assess the consequences of a single gene to the pathogenesis of DS. Using real-time RT-qPCR, we show that the human transgene is overexpressed in brains of transgenic mice. IHC and IF analysis showed a similar distribution of PEP-19 expression in wild type and transgenic mice. Basic behavioral testing of PCP4 mice revealed, in females, slight hyperactivity, decreased avoidance of open spaces, and shifted reaction to auditory stimuli - the phenotypes also seen in Ts65Dn mice carrying partial trisomy only for a subset of mouse genes homologous to HSA21. We applied Sholl analysis to dendrites from the pyramidal neurons of layer II/III from PFC and found that in PCP4 mice, the distal apical dendritic arbor has significantly more branches and intersections. Moreover, whereas wild type littermates loose dendritic length and complexity with age (5-6 months vs. 24-26 months), older PCP4 transgenic mice keep essentially the same structure of the dendritic tree from 6 to 26 months. This is the first evidence of any single DS gene, here PEP-19, altering both dendritic structure and behavior in vivo.

Meta-analysis of genome wide association studies with human height in European-originated monozygotic female twins and Northern Finland birth cohort. *J. A. Kettunen[1,2], I. Lindqvist[1], S. Ripatti[1,3], T. D. Spector[4], N. G. Martin[5], M. R. Järvelin[6,7], L. Peltonen[1,2], M. Perola[1], behalf of GenomEUtwin-project* 1) Department of Human Genetics, Wellcome Trust Sanger Institute, Cambridge, United Kingdom; 2) Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK; 3) Karolinska Institutet, Department of Medical Epidemiology and Biostatistics, Stockholm, Sweden; 4) Twin Research and Genetic Epidemiology Unit, King's College London, London, UK; 5) Genetic Epidemiology Laboratory, Queensland Institute of Medical Research, Brisbane, Australia; 6) Department of Epidemiology and Public Health, Imperial College, London, UK; 7) Department of Public Health Science and General Practice, Oulu University Hospital and University of Oulu, Finland.

In this study we report a genome wide association meta-analysis to human height. Our study aimed for reduced environmental variance in two ways: firstly by using the mean of each MZ twin pair as a phenotype and secondly genotyping a sample of individuals from an isolated area in Finland, born in the same year (1966). The sample sets comprised of 1631 European monozygotic female twin pairs from the GenomEUtwin consortium and 4734 individuals (2270 males, 2464 females) from Northern Finland Birth Cohort (NFBC) genotyped with Illumina 300 and 370 chips respectively. In the combined sample best association we observed was in the chromosome 13q14 region (p = 1.92*10-7) underlying the same area which was identified in the previously reported (Weedon, Lettre) genome wide association scans. Most of the lately reported GWA loci were replicated in our study. We performed sex specific analysis and found some interesting regions exemplified by female specific association in chromosome 5q33 region (p = 1.39*10-7). We identified some novel regions and the replication study is underway. We also tested for genes that might be responsible for increasing variance in human height. This was performed in the MZ scan and was aiming to find loci responsible for genotype-environment interaction or imprinting/gene silencing. The most significant finding for variance in stature (p = 1.22 * 10-5) was identified on 6q14 region.

Public Attitudes and Knowledge and the Integration of Genomic Medicine into Primary Care. *C. A. Christianson[1], K. P. Powell[1], S. E. Hahn[2], D. Bartz[1], T. Roxbury[1], S. Blanton[2], M. Pericak-Vance[2], V. C. Henrich[1]* 1) The University of North Carolina at Greensboro, NC; 2) The University of Miami, FL.

Integration of genomic medicine into primary care will depend in part on the publics acceptance & utilization of these services. Factors that influence the publics uptake of genetic technology include moral codes, perceived use, confidence in regulatory agencies & genetics knowledge. The purpose of this study was to assess the attitudes about genomic medicine & genetics knowledge of patients seen in three primary care practices in North Carolina & compare these to the results of a community telephone survey (N=1136). The survey was given to patients at each practice during a 3 month period. The survey included 15 attitude questions regarding genetic tests, concerns about privacy & genetic discrimination & the anticipated uses & benefits of genetic tests. There were 16 knowledge questions about family history, inheritance, screening, genetic testing, & existing privacy/discrimination laws. Most respondents (N=1183) were female (65%), Caucasian (81%) & the mean age was 57. The majority were not morally concerned about genetic testing. They were more worried about the potential loss of privacy than genetic discrimination (61% vs. 53%). They thought health insurers were more likely to use genetic information than employers (54% vs. 27%) & 69% thought the government would protect them from discrimination. Similar trends were seen on the community survey. The mean knowledge score on the patient survey (8.96, sd = 2.4) was less than on the community survey (9.44, sd = 2.1). A factor analysis identified groups of questions that measured knowledge in 5 areas. Patients knew that diseases run in families & they over estimated the utility of genetic tests. Education & experience with genetic diseases & tests were associated with higher mean knowledge scores ($p < .001$). Respondents generally approved of genetic tests, believed that genetics will positively affect healthcare & understood there is a connection between genetics & disease. These data suggest the public will support the integration of genomic medicine into primary care if their concerns about discrimination are addressed.

Homologous Recombination conserves DNA sequence integrity throughout the cell cycle in embryonic stem cells.
L. Serrano, L. Liang, Y. Chang, L. Deng, C. Maulion, S. C. Nguyen, J. Tischfield Genetics, Rutgers University, Piscataway, NJ.

The maintenance of genomic integrity is crucial to embryonic stem cells (ESCs) considering the potential for propagating undesirable mutations to the resulting somatic and germ cell lineages. Indeed, we previously reported that mouse ESCs exhibit a significantly lower mutation frequency compared to differentiated cells. This could be due to more effective elimination of genetically damaged cells via apoptosis, or especially robust, sequence-conserving DNA damage repair mechanisms such as homologous recombination (HR). We used fluorescence microscopy and 3D quantitative image analysis to compare mouse ES and differentiated cells, with regard to HR-mediated repair of spontaneous and x-ray-induced double strand breaks (DSBs). Microscopic analysis of repair foci and functional assay of nuclear extracts both indicate that HR is greater in mouse ESCs compared to fibroblasts. Strikingly, HR is not exclusively associated with post-replicative chromatin and appears to repair induced or spontaneous DNA damage throughout the ESC cell cycle, including in G1 synchronized cells. This is dramatically different from what we observe in primary fibroblasts, and from what has been reported in other adult mammalian cell types, where HR is essentially confined to S-phase. This result suggests that alternative templates, such as homologous chromosomes, are more frequently used to repair DSBs in ESCs, which can result in a crossover and potentially lead to loss of heterozygosity (LOH). However, our data indicate that much of the HR occurs before DNA replication. In this way ESCs would maintain a low mutation frequency through an error-free DNA repair pathway (HR), which would alter linkage relationships compared with parental cells but does not lead to LOH. Relatively frequent HR utilizing homolog chromosome sequences preserves genome integrity in ESCs and has distinctive and important genetic consequences to subsequent somatic and germ cell lineages.

Balanced t(12:17) translocation upstream of SOX9 resulting in 46,XX testicular DSD. *O. M. Refai[1], A. Friedman[1], L. Terry[2], T. Jewett[2], H. Otsrer[1]* 1) NYU School of Medicine, New York, NY; 2) Wake Forest School of Medicine, Winston Salem, NC.

Individuals with rare cytogenetic variants have contributed to our understanding of the genetics of sex development and its disorders. Study of such individuals has demonstrated the dose-dependency of SOX9 in gonadal development. Here, we report a case of a balanced chromosome 12;17 translocation 46,XX,t(12;17)(q14.3;q24.3) resulting in 46,XX testicular DSD in one of monozygotic twins concordant for genotype and phenotype. The translocated chromosomes were isolated via human-hamster somatic cell hybridization and screened using microsatellite genotyping. A pair of cell lines containing both the normal chromosome 12 as well as the 12;17 translocation were analyzed on Affymetrix 500K SNP arrays. The data were analyzed for copy number and loss of heterozygosity to localize the breakpoints on both chromosomes. The chromosome 12 breakpoint occurred at 64.4-64.6 Mb, i.e. within intron 2 of the HMGA2 gene. The chromosome 17 breakpoint occurred at 66.4-67.1 Mb, at least 400 kb upstream of SOX9, and near the well-characterized promoters 300 kb upstream of the first exon of this gene. Thus, the t(12;17) translocation disrupted the HMGA2 gene, potentially causing it to up-regulate the testis-determining SOX9 gene during gonadal development and the phenotype of 46,XX testicular DSD.

A Common Genomic Copy Number Variant Locus Is Associated with Colorectal Cancer Risk and Poor Survival.

R. Abdel-Rahman[1], G. Zogopolous[3,4], S. Farrington[2], A. Gropper[3,4], K. Ha[3,4], K. hyeja[5], S. Moore[3,4], G. Bader[5], R. gryfe[3,4], B. Zanke[6], A. Montpetit[7], P. Laflamme[7], J. Hurley[8], T. Chiang[7], A. Tenesa[2], D. Farhat[2], T. Hudson[7], M. Dunlop[2], G. Steve[3,4], H. Campbell[1,2] 1) Public Health, Univ Edinburgh, Edinburgh, United Kingdom; 2) Institute of genetics and molecular medicine; 3) Samuel Lunenfeld Research Institute; 4) Fred Litwin Centre for Cancer Genetics; 5) University of Toronto; 6) Cancer care Ontario; 7) Ontario Institute for Cancer Research; 8) biotrove inc.

Background: Genomic copy number gains and deletions are increasingly recognized as underlying disease susceptibility. Methods: Independent genome-wide association studies were conducted concurrently in Canada and Scotland to investigate the role of copy number variation (CNV) in colorectal cancer. 1257 cases and 1336 controls from Canada and 1012 cases and 1012 controls from Scotland were assayed for CNVs across 500K and 550K genomic markers respectively. Results: Genomic copy number gain at one locus was found to be associated with increased risk of colorectal cancer (minimum p value $1.1x10^{-}9$ in the Canadian study and $p=1.9 \times 10^{-}11$ in the Scottish study). These observations were verified using quantitative PCR in an independent series of 304 cases and 354 controls. In this series, the locus copy number gain was detected in 13.9% cases versus 3.5% controls ($p=1.46x10^{-}5$; O.R.=3.95) and was associated with reduction in overall cancer survival (multivariate Cox proportional hazard ratio = 1.7 [95% CI 1.3 - 2.2], $p=6x\ 10^{-}5$). Five-year survival was 57% in carriers of this copy number gain, compared to 71% in non-carriers. Conclusions: We report the first association of a common copy number change with increased cancer risk in individuals without a familial cancer syndrome. Copy number gain at the identified locus is associated with the risk factor for colorectal cancer, and an independent prognostic predictor of worse survival. The identified locus is located near gene that provides a plausible candidate that might mediate risk and survival effects. These findings have clinical relevance and implications for predicting risk within populations.

Weighted Genome-Wide Association Study of Bipolar Disorder. *M. B. McQueen[1], P. Sklar[2], J. W. Smoller[2], J. Fan[2], I. Ionita-Laza[3], V. L. Nimgaonkar[4], S. V. Faraone[5], S. M. Purcell[2], B. Devlin[6], N. M. Laird[3]* 1) Inst Behavioral Genetics, Univ Colorado, Boulder, CO; 2) Center for Human Genetic Research, Mass General, Boston, MA; 3) Department of Biostatistics, Harvard School of Public Health, Boston, MA; 4) Departments of Psychiatry and Human Genetics, University of Pittsburgh, Pittsburgh, PA; 5) Department of Psychiatry and Behavioral Sciences, Upstate Medical University, State University of New York, Syracuse, NY; 6) Department of Psychiatry, University of Pittsburgh, Pittsburgh, PA.

We recently conducted two large-scale gene-mapping efforts targeting bipolar disorder. The first was a combined analysis of eleven linkage studies including over 1000 families (McQueen et al, Am J Hum Genet 2005). The second was a genome-wide association study (GWAS) obtained through the Systematic Treatment Enhancement Program for Bipolar Disorder (STEP-BD) involving 955 bipolar I cases and 1498 controls (Sklar et al, Mol Psychiatry 2008). In an effort to combine evidence across both strategies, we adopted the weighted hypothesis testing approach as implemented by Roeder et al (Am J Hum Genet, 2005). In particular, we used the genome-wide linkage statistics (Z-scores) from the combined linkage scan to weight the GWAS p-values. In this way, association signals arising from prior linkage regions are "up-weighted" while signals from other regions are "down-weighted". By adopting this approach, the same SNP identified in the Sklar et al study (rs4939921; chromosome 18) was the most significant signal, even though chromosome 18 displayed only modest linkage evidence in the combined analysis. Furthermore, as expected, SNPs with modest statistical significance residing in the chromosomes 6q, 8q and 9p linkage regions achieved much stronger statistical evidence by incorporating the prior linkage information. Therefore, while there is some overlap with respect to the "top" association signals using both the weighted and unweighted GWAS, there are a considerable number of SNPs identified using the weighted approach that warrant follow-up.

Optimization of the NimbleGen array Comparative Genomic Hybridization Protocol. *M. Ikeda, S. T. Warren*
Human Genetics, Emory Univ, Atlanta, GA.

To improve identification of genomic deletions or duplications, we sought to modify the current protocol for NimbleGen high-density array Comparative Genomic Hybridization. Hypothesizing that more stringent hybridization and wash conditions would increase our ability to detect copy number changes, we investigated three different labeling protocols and two different hybridization and wash systems. We tested labeling protocols using Cy3/Cy5-end labeled randomers (NimbleGen strategy), Cy3/Cy5-labeled nucleotides, or amine-modified nucleotides (AM) to which Cy3/Cy5 could be conjugated. While NimbleGen recommends the Maui hybridization system and manual washes, we also tested the Tecan HS4800Pro which allows greater user control of hybridization conditions. Using a sample with a validated deletion, we tested various strategies. Following the NimbleGen protocol, for this sample, the average log2 for deletions was -0.51 and 0.66 for duplications. We first increased hyb temp from 42C to 52C, which decreased the average log2 by 56% for deletions and 50% for duplications. Substituting AM nucleotides, the average log2 for deletions increased by 2% while duplications decreased by 15%. By increasing the temp and changing the label, the average log2 values increased deletions by 49% and decreased duplications by 22%. With the Tecan station, we used more washes at higher temps, and tested hybridization at 42C and 52C with increased agitation. These factors increased discrimination for the NimbleGen labeling strategy at both the original and increased hyb temp. At 42C, the average log2 increased for deletions by 35% and for duplications by 45% and, at 52C, 124% and 1% respectively. Finally, we explored alternative labels at 52C. While the Cy3 or Cy5-labeled nucleotides decreased the average log2 for deletions by 53% and duplications by 5%, the AM nucleotides increased the average log2 for deletions by 193% and 22% for duplications. By using AM-based labeling, increasing hybridization temp and increasing stringency of the washes, we have demonstrated a substantial increase in the average log2 values for deletion and duplications on NimbleGen arrays. These protocol changes allow greater certainty in the identification of copy number variant loci.

A Polymorphism in *SLC34A2*, a strong positional and biological gene for osteoporosis, is associated with bone mineral density in three independent populations. *J. Liu[1], N. Hoppman-Chaney[2], P. Giampietro[3], C. McCarty[3], B. Mukesh[3], L. Ivacic[3], N. Ghebranious[3], E. A. Streeten[1], D. McBride[1], A. R. Shuldiner[1], B. D. Mitchell[1]* 1) Dept Human Genetics, Univ Maryland, Baltimore, MD; 2) Clinical Molecular Genetics, Mayo Clinic, Rochester, MN; 3) Dept Med Genetics, Marshfield Clinic, Marshfield, WI.

Low bone mineral density (BMD) is a major risk for osteoporosis and is highly heritable. In a previous linkage study carried out in Mexican Americans, strong evidence was obtained for a QTL influencing forearm BMD to a region harboring over 30 known genes on chromosome 4p near D4S2639 (LOD= 4.3). To identify gene(s) on 4p accounting for this linkage, we genotyped 1506 SNPs in or near every gene within the 2-LOD support interval. We observed that 6 SNPs from four genes (*KCNIP4*, *SLC34A2*, *LDB2* and *STIM2*) were strongly associated with forearm BMD ($p<0.001$). Of these, one SNP, rs2240997 (MAF=0.26), in *SLC34A2* showed the strongest association with both forearm ($p=0.0003$) and hip BMD ($p=0.000035$). The at-risk allele was associated with an estimated 0.27 unit decrease in Z score of hip BMD and accounted for 3% of variance in this trait. These six SNPs were genotyped in an independent population comprising postmenopausal Caucasian women without osteoporosis or osteopenia (n=293). Rs2240997 (MAF=0.14) was strongly associated with decreased Z score of BMD ($p=0.006$) in this replication set with the risk allele associated with a 0.23 unit decrease in BMD Z score, consistent with our original finding in Mexicans Americans. As a second replication, rs2240997 was associated with spine BMD in a recent published BMD genome-wide association study of Icelanders (n=5861; $p=0.027$) (Stefansson et al, N Engl J Med; 2008; 358). In summary, we have identified a genetic variant in *SLC34A2* that influences BMD in Mexican Americans and observed this association to be replicated in two independent Caucasian populations. SLC34A2 is a sodium-phosphate transport protein expressed mainly in the intestine, which may be involved in regulation of bone metabolism by affecting phosphate absorption, making it an excellent biological candidate gene for BMD and osteoporosis.

SNPs in *FTO* and *GNB3* are associated with obesity in Mexicans. *D. Velazquez[1,2], I. Silva-Zolezzi[1], A. Contreras[1], J. S. Hernandez[1], C. Diaz[1], R. Gutierrez[1], K. Carrillo[1], R. Reynoso[2], M. F. Herrera[2], E. Garcia-G[2], G. Jimenez-Sanchez[1]*
1) National Institute of Genomic Medicine, Mexico; 2) National Institute of Medical Sciences, Salvador Zubiran, Mexico.

Obesity is a worldwide epidemic. In Mexico, approximately one fourth of the population is obese (BMI 30 kg/m2). We designed a case-control study in Mexicans by their BMI (30 and 27 kg/m2 respectively). A total of 138 cases and 92 non-obese controls were included (mean BMISD in cases=26.74.3 kg/m2 and in controls=46.810 kg/m2, p<0.0001). About 50% of cases reported to have a first-degree obese relative and/or to have obesity since childhood. We compared these groups with 1288 individuals obtained from the general population (from 8 different states of Mexico). We genotyped the following 23 SNPs in 14 genes by TaqMan (ABI, USA): *ADIPOQ* (rs1501299, rs2241766, rs822396), *LEPR* (rs1137100, rs1137101, rs8179183, rs13306526), *LEP* (rs17151919), *GNB3* (rs5443), *MC3R* (C_27859133), *MC4R* (rs13447335, rs13447324, rs13447332), *NR3C1* (rs6195, rs33391, 6192), *AGRP* (rs5030980), *ADRB3* (rs4994), *ADRB2* (rs1042714), *PPARG2* (rs1801282), *POMC87* (rs1042571), *POMC158* (rs2071345) and *FTO* (rs8050136). To test for association we used traditional Chi square analysis and Cochran-Armitages trend test. Our results showed association for rs5443 in *GNB3* (OR=1.7 95% CI=[1.2-2.6], p=0.007) and for rs8050136 in *FTO* (OR=2.1 95% CI=[1.3-3.4], p=0.002). Observed MAF in cases for selected SNPs were closer to those in control population particularly in the case of *GNB3*. In addition, we observed that females were predominantly more obese than males (p=0.0001), and both, fat percentage and waist & hip ratio were significantly different between the groups (p<0.0001). We found evidence that variants in *GNB3* and *FTO* are moderately associated to obesity in Mexican Mestizos. To increase statistical power in our study we are increasing sample size and will further analyze population stratification in our sample.

Phagocytosis is altered in cell cultures from patients with choroideremia. *A. K. Thiagarajasubramanian[1], N. Strunnikova[1], Y. Sergeev[1], N. Gordiyenko[1], C. Silvin[2], R. Fariss[3], I. M. MacDonald[1]* 1) OGFVB/ NEI/NIH, Bethesda, MD; 2) NHGRI/NIH, Bethesda, MD; 3) OSD/NEI/NIH, Bethesda, MD.

Choroideremia (CHM) is an X-linked form of retinal degeneration characterized by slowly progressive atrophy of the retinal pigment epithelium (RPE), photoreceptors, and choroid. The disorder is caused by loss-of-function mutations in the Rab Escort Protein 1 gene (*REP1*), which is a key regulator of vesicular trafficking, phagosome fusion and maturation. CHM is thought to specifically affect the eye as, in other tissues in the body, a second Escort Protein (REP-2) may compensate for the functional absence of REP-1. To test the potential systemic effect of REP1 mutations, we performed experiments on skin fibroblasts and monocytes from CHM patients. All patients had mutations in *REP1* and lacked the protein product as determined by immunoblot analysis with anti-REP-1 antibody. Phagocytosis in fibroblasts was induced with collagen-coated FluroSpheres (Invitrogen, Carlsbad, CA). Similar experiments were undertaken in fibroblasts and monocytes with *E. coli* conjugated with a pH dependent dye (pHrodo). Phagocytosis was tracked using fluorescence-activated cell sorting (FACS) and live cell imaging analysis. Skin fibroblasts from 3 CHM patients and age-matched controls, and monocytes (CD14 positive fraction) from 6 CHM patients and controls were compared for differences in phagocytosis. In the fibroblasts from CHM patients, the rate and quantity of *E. coli* pHrodo uptake was decreased compared to the controls. The same effect was seen in fibroblasts from CHM patients fed with collagen microspheres. In the monocytes from CHM patients, *E. coli* pHrodo uptake was significantly increased in comparison to the controls. These novel findings from the study of monocytes and fibroblasts suggest a systemic dysregulation of phagocytosis in CHM patients. These observations may lead to new approaches to the study and potential treatment of this unique disorder.

Expression microarray platform performance and content analysis in mouse model of mitochondrial disease therapy. *E. F. Rappaport[1], Z. Zhang[1], M. J. Falk[2], D. L. Gasser[3]* 1) Biomedical Informatics, Children's Hospital of Philadelphia, Philadelphia, PA; 2) Division of Human Genetics, Children's Hospital of Philadelphia, Philadelphia, PA; 3) Department of Genetics, University of Pennsylvania School of Medicine.

As more options for microarray platform emerge, questions arise concerning array content and design to best maximize experimental data analysis. Prior cross-platform studies have tended to provide a more global comparison of array performance. Our goal was to compare the functional consequences of differences in array data between platforms utilizing samples for a discrete animal model in which we had already established a foundation of biological knowledge. Liver RNA was isolated from four liver-conditional Pdss2 knockout mice aged 140 to 169 days (B6. Alb/cre,Pdss2loxP/loxP), two of whom were treated with the antioxidant Probucol since birth and the other two of whom were untreated. Targets from these samples were prepared and hybridized to Affymetrix Mouse Genome 430_2 (a 3 focused array), Affymetrix Mouse Exon 1.0 ST, and Illumina Mouse Whole Genome WG-6 arrays. Significant inter-platform correlation was seen in differential gene expression between treated and untreated groups, and all three platforms identified Biosynthesis of Steroids as the most altered KEGG pathway. However, the three platforms differed in terms of content, annotation, and software support. The exon array had superior representation by covering more than 98% of KEGG genes, but biased results in favor of bigger genes. The 3-focused array had superior software and well-established analysis methods, but some outdated content and less extensive coverage than the other two platforms. The Illumina platform demonstrated high technical repeatability, up-to-date content, and less-ambiguous annotation, but software and analytic methods were not as extensive as for the 3-focused arrays. These results indicate that similar data and conclusions can be generated with any of these expression array platforms, but the specific goals of the investigator might determine which one is best suited to a given study.

Markers for sex-specific prenatal selection. *E. Ucisik-Akkaya, C. Davis, B. Morrison, T. Do, M. T. Dorak* Genomic Immunoepidemiology, HUMIGEN LLC, the Institute for Genetic Immunology, 2439 Kuser Road, Hamilton, NJ 08690.

There are no established genetic markers mediating prenatal selection. Most studies are designed to find clues focused on couples experiencing recurrent miscarriages, but such events represent only a fraction of total prenatal loss. Based on the strikingly high male-to-female ratio at the time of fertilization, which diminishes by birth, we postulated that prenatal selection is sex-specific and strongly acts against males. To test this hypothesis, we examined candidate genes in male and female newborns (n=389). We first considered the HLA complex to replicate previously reported findings. We identified a haplotype (HSPA1B rs1061581- HLA-DRA rs7192- HLA-DQA1 rs1142316) whose major alleles characterize the ancestral HLA-DRB4 lineage (HLA-DR4, DR7 and DR9), while the minor alleles represent the HLA-DRB3 lineage (DR3, DR11/12 and DR13/14). Similar to our previous finding by HLA-DRB1 typing, males had a deficit for homozygosity for the SNP haplotypes corresponding to the ancestral lineages (6.7% vs 16.4%; $P = 0.007$). These SNPs can replace HLA typing in replication studies. In KLRK1 (chromosome 12) encoding the natural killer receptor NKG2D, multiple SNPs showed reduced heterozygosity rates in males compared with females ($P < 0.05$). Hardy-Weinberg equilibrium was violated in boys but not in girls. Wrights fixation index suggested the loss of up to 18.4% of males during the prenatal period. Given the eminent role played by NK cells and in particular the NKG2D receptor in feto-maternal interactions, this finding is biologically plausible. In contrast to other reports, we did not find any evidence for the involvement of TP53 rs1042522 (R72P) in prenatal selection. Heme oxygenase 1 activity is implicated in fetal protection and we found evidence for decreased minor allele homozygosity at HMOX1 SNP rs2071748 in males (13.8 vs 22.6%; $P = 0.04$). Overall, these findings suggested that male-female differential in genotype frequencies at loci relevant to fetal survival may be sought to discover markers for prenatal selection.

Promoter polymorphisms of the *CSF2* gene are associated with its transcription. *JQ. He, J. Yuan, P. Paré, A. Sandford* James Hogg iCAPTURE Centre for Cardiovascular and Pulmonary Research, St. Pauls Hospital, University of British Columbia, Vancouver, BC, Canada.

Background: We previously reported an association of the A allele of a single nucleotide polymorphism (SNP) of *CSF2*_545A/G (-1440A/G, rs2069616) with atopic asthma in at-risk children at 1 year of age. A later report showed that the mutant allele (C allele) of another SNP, *CSF2*_-677A/C (rs1469149), in complete linkage disequilibrium (LD) with the -1440A/G SNP, was associated with lower frequency and severity of childhood allergic dermatitis. We hypothesized that promoter polymorphisms of *CSF2* are associated with its transcription. Methods: A third SNP (*CSF2*-1916T/C, rs2069614), in complete LD with the two abovementioned SNPs, was identified from the SeattleSNP database. Two constructs containing the wild-type haplotype of these 3 SNPs (-1916T/-1440A/-677A) and the mutant-type haplotype (-1916C/-1440G/-677C) were obtained from PCR amplifying samples homozygous for either the wild-type or mutant-type at all three SNPs. The effect of these SNPs on gene expression was evaluated by the transient expression of a luciferase reporter gene using the pulmonary epithelial cell line A549. Results: The transcriptional activity of the mutant-type *CSF2* haplotype (CGC) was significantly decreased compared with the wild-type *CSF2* haplotype (TAA) (14% decrease, $p = 0.0002$). Stimulation by TNF increased the transcriptional activities by 1.6 fold. However, the fold increases of transcriptional activities after TNF stimulation were not significantly different in cells with the wild-type vs. mutant-type haplotype. Conclusion: Consistent with the previous association study that the mutant-type of *CSF2* genotypes were associated with protection against allergic diseases, the mutant-haplotype was associated with less transcription in reporter gene assays. Supported by the Canadian Institutes of Health Research.

Feline Acute Intermittent Porphyria (AIP) Masquerading as Congenital Erythropoietic Porphyria: Biochemical & Molecular Studies of Three Unrelated Cats. *S. Clavero[1], D. F. Bishop[1], M. Haskins[2], U. Giger[2], R. J. Desnick[1]* 1) Genetics & Genomic Sciences, Mount Sinai School of Medicine, New York, NY; 2) Section of Medical Genetics, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA.

Acute Intermittent Porphyria (AIP) is an autosomal dominant inborn error of heme biosynthesis, due to the half-normal activity of hydroxymethylbilane synthase (HMBS). Humans with AIP can experience life-threatening acute neurologic attacks precipitated by various drugs, dieting and hormonal changes. No naturally occurring animal models exist. Three unrelated domestic shorthair or Siamese cats from three disparate locations in North America were referred for evaluation of congenital erythropoietic porphyria (CEP) due to reddish-colored urine and reddish-brown teeth (erythrodontia) that fluoresce under UV light. Although the clinical phenotype was consistent with recessively inherited CEP, inheritance appeared autosomal dominant and sequence analysis of the feline uroporphyrinogen III synthase gene identified no mutations. Analysis of urinary porphyrins and metabolites revealed marked elevations of PBG (2- to 7-fold), uroporphyrinogen I (25- to 101-fold) and III (42- to 149-fold), coproporphyrinogen I (3- to 12-fold) and III (3 to 6-fold), and normal to 8-fold elevated 5-aminolevulinic acid (ALA), suggesting an acute porphyria. Erythrocytes from all three cats had ~50% HMBS activity, consistent with AIP. Cloning and sequencing of the feline *HMBS* gene from these three cats revealed three different mutations: an in-frame three base deletion (c.842_844delGAG), reported in a human AIP family, a single thymidine insertion (c.189insT) and a missense mutation (R149W; R149X in human AIP). While these AIP cats phenotypically masquerade as CEP, they are actually AIP, with the more severe deletion and insertion mutations resulting in constitutively high ALA and PBG levels, indicating that they have a cronic acute attack syndrome. These cats are the first natural animal models for AIP and future studies of their neuropathology may provide insights into the neurotoxic metabolites causing human AIP. Support: NIH R01DK026824 & RR02512.

Next-Generation whole genome sequencing of a single human recapitulates population signatures of natural selection. *F. C. L. Hyland[1], Z. Zheng[1], S. McLaughlin[2], E. F. Tsung[2], H. Peckham[2], C. Lee[2], G. Costa[2], F. M. De La Vega[1], K. McKernan[2]* 1) Applied Biosystems, Foster City, CA; 2) Beverly, MA.

We sequenced the whole genome of an African Yoruba individual (in HapMap and 1000 Genomes Projects) by sequencing fragment and mate-pair libraries of various sizes with the Applied Biosystems SOLiD system. We detected over 2,000,000 SNPs; 81.4% are in dbSNP. SNPs are under-represented in exons (1.98%). 11% of exons contain at least one SNP. Of coding SNPs, 54% are silent, 45% are missense, and 0.6% are nonsense. We categorize the functions of genes using the Panther ontology, and we annotate the damaging potential of non-synonymous SNPs (nsSNPs) using PolyPhen. 20.5% of nsSNPs in this sample are predicted to be damaging, vs 33.2% in the PolyPhen database. We discovered that transcription factors, ligases, growth factors, receptors, and RNA helicases are the molecular functions most under-represented for damaging mutations. Further, we discovered that GPCR genes involved in Olfaction, and genes for Immunity and defense are the biological functions most highly over-represented for damaging mutations. Olfaction and Immunity have previously been observed to be under recent positive selection in human populations; we observe evidence of purifying selection and of directional selection in a single human sample. We performed gap alignments of reads looking for small InDels (11bp deletion to 4bp insertion), identifying 90,000 small InDels. The number of insertions and deletions is symmetrical; 1bp is the most common size. InDels are significantly underrepresented in exons. Most InDels in exons occur in the last exon, which contains the 3 UTR region; they are also overrepresented in the first exon, containing the 5 UTR - as expected, since an InDel in a translated region may cause a frameshift. Of 1290 exons which contain an InDel, 979 are the first or last exons (851 are the last exon). InDels are highly non-randomly distributed within genes: we expect to see 13 genes with 2 or more InDels, instead we see 184 ($p << 10\text{-}10$). These observations are consistent with a low rate of false positives, and provide evidence for strong purifying selection against InDels in translated exons.

Genome-wide linkage analysis of an autosomal recessive hypotrichosis identifies a novel P2RY5 mutation. *L. Petukhova[1], E. C. Sousa, Jr.[2], A. Martinez-Mir[1], A. Vitebsky[1], L. G. dos Santosc[3], L. Shapiro[4], C. Haynes[5], D. Gordon[6], Y. Shimomura[1], A. M. Christiano[1, 7]* 1) Department of Dermatology, Columbia University, New York, NY, USA; 2) Departmento de Clínica Geral (Clínica Cirúrgica I), Universidade Federal do Piauí, Teresina, Piauí, Brazil; 3) Departamento de Pathologia, Universidade Federal do Piauí, Teresina, Piauí, Brazil; 4) dDepartments of Biochemistry & Molecular Biophysics and Ophthalmology, Columbia University College of Physicians & Surgeons, New York, New York, USA; 5) Laboratory of Statistical Genetics, Rockefeller University, New York, NY, USA; 6) Department of Genetics, Rutgers University, Piscataway, NJ, USA; 7) Department of Genetics & Development, Columbia University College of Physicians & Surgeons, New York, New York, USA.

While there have been significant advances in understanding the genetic etiology of human hair loss over the previous decade, there remain a number of hereditary disorders for which a causative gene has yet to be identified. We studied a large, consanguineous Brazilian family that presented with sparse woolly hair at birth that progressed to severe hypotrichosis by the age of 5, in which 6 of the 14 offspring were affected. After exclusion of known candidate genes, a genome-wide scan was performed to identify the disease locus. Autozygosity mapping revealed a highly significant region of extended homozygosity (LOD score of 10.41) that contained a haplotype with a linkage LOD score of 3.28. Results of these two methods defined a 9 Mb region on chromosome 13q14.11-q14.2. The interval contains the P2RY5 gene, in which we recently identified pathogenic mutations in several families of Pakistani origin affected with autosomal recessive woolly and sparse hair. After the exclusion of several other candidate genes, we sequenced the P2RY5 gene and identified a homozygous mutation (C278Y) in all affected individuals in this family. Our findings show that mutations in P2RY5 display variable expressivity, underlying both hypotrichosis and woolly hair, and underscore the essential role of P2RY5 in the tissue integrity and the maintenance of the hair follicle.

Increase in Left Ventricular Mass in Glycogen Storage Disease IIIa. *S. M. Vertilus[1], S. Austin[2], K. Foster[1], K. Boyette[2], D. Bali[2], J. S. Li[1], P. Kishnani[2], S. Wechsler[1,2]* 1) Pediatric Cardiology, Duke University Medical Center, Durham, NC; 2) Medical Genetics, Duke University Medical Center, Durham, NC.

Background: Glycogen Storage Disease type IIIa (GSD IIIa) is caused by a deficiency in glycogen debranching enzyme resulting in an accumulation of glycogen in the liver, skeletal and cardiac muscle. Some patients (pts) with GSD IIIa develop an increase in left ventricular mass (LV mass) by echocardiography, but the rate of increase and clinical significance remain unknown. There are a few reports of sudden cardiac death and one reported case of heart, liver, and kidney transplant. To date, there is no systematic review of long term follow up to assess the extent and rate of progression. Methods: We evaluated a cohort of 12 pts with GSD IIIa, mean age 12.9 years (5 months - 39 yrs), over a mean time period of 4.8 yrs (1.1 - 9.1 yrs). GSD IIIa was confirmed by genetic and/or enzyme analysis in all pts. These pts had serial echos read by two investigators (SV, KF). Measurements reviewed included LV mass, shortening fraction (SF), and ejection fraction (EF). No alternate cause of increased LV mass was identified in any pt. Results: Eight of the 12 pts had an increase in LV mass from baseline to endpoint assessments. Four of these 8 pts had a substantial increase in mass over time. Three patients doubled their LV mass over time. Two pts had rapid increases in LV mass (40 gm/m2 and 24 gm/m2) over a short period (1 - 1.5 yrs.). The mean LV mass at baseline was 54.8g/m2 (30 - 103) and the mean LV mass at endpoint was 74.2g/m2 (43 - 129). For all 12 pts, the mean change in LV mass was 19 g/m2 (-13 - 75), from baseline to endpoint echos. SF and EF remained normal for all patients at all points examined (36 and 24 measurements respectively) over the entire period. No pt required cardiovascular medications. Conclusion: Pts with GSD IIIa develop an increase in LV mass, albeit not to the same extent as seen in patients with other types of GSDs, but with no diminution in SF or EF by echo. This suggests that serial echos with attention to left ventricular mass are necessary in the care of these pts.

DOES THE NEURAL RESTRICTIVE SILENCER FACTOR (NRSF) REGULATE THE EXPRESSION OF CHOLINE ACETYLTRANSFERASE (CHAT) IN CEREBRAL TISSUE OF ALZHEIMER'S DISEASE PATIENTS? *R. E. Gonzalez-Castañeda[1,2,4], V. Sánchez-González[2,4], V. Loera-Castañeda[5], G. Vázquez-Camacho[3], M. Macías-Islas[3], A. Barba-González[4], G. Gabriel Ortiz[2,4]* 1) Neurociencias, Universidad de Guadalajara, Mexico; 2) Laboratorio de Desarrollo-Envejecimiento; Enfermedades Neurodegenerativas, CIBO, IMSS,; 3) Depto. de Anatomía Patológica y Depto. de Neurología, UMAE-HE, CMNO, IMSS, México; 4) Depto. de Ciencias Básicas Médicas, Instituto Tecnológico y de Estudios Superiores de Monterrey, Guadalajara, México; 5) Centro Universitario de Investigación Integral Regional, Unidad Durango.

Alzheimer's disease (AD) is a particular form of progressive dementia associated with distinct neuropathologic changes. Likewise, the most consistent biochemical changes is a decrease in choline acetyltransferase (CHAT) enzyme activity in the target areas of basal forebrain cholinergic neurons. Previous reports showed that the syntheses of the cholinergic neuron-essencial molecules involved in cholinergic neurotransmission are elaborately controlled by DNA regulatory elements like the neural restrictive silencer factor (NRFS). Objective. To evaluate ChAT and NRSF genetic and protein expression cerebral tissues of AD patients. Methods. Frontal, temporal, entorhinal and parietal cortices were obtained from four autopsied patients with Alzheimer's, and patients with no clinical history or pathological findings suggestive of neurological diseases. The cerebral tissue was processed by the guanidine isothiocyanate method for RNA extraction. ChAT and NRSF expression was determined by reverse transcription-polymerase chain reaction (RT-PCR) and Western blotting. Results. CHAT expression in the control and AD group is greater in the frontal and entorhinal cortices and smaller in the temporal and parietal regions. Nevertheless, the expression is greater in the control than in the AD group. Despite the CHAT variations in the control group, the levels of ChAT protein remain constant; whereas in the AD group the expression presents a peak in the entorhinal cortex and a remarkable diminution in the frontal region ($p>0.05$, W test). The regional analysis of the NRSF gene is greater in the AD group ($p>0,05$, W test).

Joint effects of circadian genes and serum androgens on prostate cancer risk in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. *L. W. Chu[1], Q. Li[1], K. Yu[1], Y. Zhu[2], W.-Y. Huang[1], J. M. Weiss[1], P. S. Rosenberg[1], I. Menashe[1], S. Quraishi[1], A. P. Chokkalingam[3], R. B. Hayes[1], S. J. Chanock[1], A. W. Hsing[1]* 1) NCI, NIH, Bethesda, MD; 2) Yale University, New Haven, CT; 3) University of California, Berkeley, CA.

Circadian rhythms are controlled endogenously by 9 circadian genes and can influence androgen biosynthesis, which is important for prostate development. Using data from a case-control study nested in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial used for the NCI Cancer Genetic Markers of Susceptibility Project (283 non-aggressive and 236 aggressive incident cases, and 563 controls), we examined the joint effects of circadian genes (represented by 155 SNPs) and serum androgens (categorized by quartiles, Q1-4) on prostate cancer risk. Polytomous regression analysis and likelihood ratio tests (LRT) showed significant interactions between androgens and 40 SNPs in 9 circadian genes(p_{LRT}<0.05). For example, for non-aggressive cases, the *CSNK1E* rs135757 A allele was not associated with prostate cancer risk relative to the GG genotype among men with Q1 levels of 3-androstanediol glucuronide (3-diol G; a surrogate marker for tissue androgen). However, among men with Q2 and Q3 levels of 3-diol G, the GA genotype conferred 1.5- and 1.7-fold increased risks, respectively (95% CI, 0.9-2.5 and 1.0-2.8, respectively), and the AA genotype conferred 3-fold increased risks (95% CI, 1.3-6.6 and 1.3-6.9, respectively; $p_{interaction}$=*0.003*); results for Q4 are less stable due to small numbers. After adjusting for all SNPs tested within the same gene by bootstrapping, significant interactions remained between androgens and 6 SNPs in 4 circadian genes, including that between 3-diol G and *CSNK1E* rs135757 (p_{LRT}=*0.03*). The truncated product method (TPM) for assessing pathway effects further showed significant interactions between the circadian pathway (including all SNPs from the 9 genes) and 3-diol G (p_{TPM}=*0.048*) and total testosterone (p_{TPM}=*0.03*). These results suggest that genes in the circadian pathway may interact with androgens to modulate prostate cancer risk.

Targeted genomic sequencing at an association locus. *A. Kapoor[1], D. Arking[1], A. OConnor[1], M. Sosa[1], N. Hansen[2], M. Ross[3], D. Bentley[3], J. Mullikin[2], A. Pfeufer[4], N. I. S. C. Comparative Sequencing Program[2], A. Chakravarti[1]* 1) IGM, JHU, Baltimore, MD; 2) NHGRI, NIH, Bethesda, MD; 3) Illumina, Little Chesterford, UK; 4) TU Munich, Germany.

Genome-wide association studies typically reveal a 15-150 kb locus with common variants that confer disease-susceptibility. There are yet no validated methods for identifying susceptibility variants directly, however, DNA sequencing to reveal all extant variation of target locus is a necessary first step. We describe a method to recover and sequence the entirety of 141 kb of NOS1AP locus associated with QT-interval. The target locus comprises of 5 region, exon 1 and 2, intron 1 and most of intron 2 of NOS1AP, and has 554 dbSNP/273 HapMap SNPs. DNA from target interval was recovered by long-range PCR and pooling of 16 overlapping amplicons. One amplicon showed differential allele-specific amplification that we mapped to a 1051 bp segment arising from sequence variation at a repeat motif on a haplotype marked by the A allele at rs4656349: we amplified this separately. We pooled all amplicons per sample from 8 CEU and 8 YRI HapMap samples and 48 samples from individuals with extreme QT-intervals. Direct sequencing of amplicon pool was performed using Solexa SBS Technology and paired-end reads of 25 nt were aligned to a reference sequence (NCBI36). Data were analyzed using ELAND and MPG software and we present the findings from CEU samples. The mean coverage depth in reads ranged between 269-555 (ELAND) and 304-792 (MPG) with variation largely from amplicon-to-amplicon differences and at an ELAND threshold of 6 (2 PhredQ30 bases or 3 Q20 bases) missed coverage of only 69-593 bp of the locus. MPG detected 535 known and 127 novel SNPs. There was 98.9% concordance of genotypes with HapMap, with all discrepancies at 11 positions due to genotyping errors from calls on complementary strands or call reversals and at 2 positions from deeper Solexa coverage. These results demonstrate the high specificity and sensitivity of this approach for obtaining full-coverage and a complete inventory of all genetic variation in a genomic region. Analyses of structural variation and additional samples is under completion.

A genome-wide survey of recent natural selection in African-origin samples. *T. Feng[1], H. Lyon[2], B. Tayo[3], S. J. Kang[1], C. Chiang[2], R. Cooper[3], J. Hirschhorn[2,4], X. Zhu[1]* 1) Dept. of Epi & Biostat, Case Western Reserve Univ., USA; 2) Div. of Genetics, Childrens Hospital Boston and Harvard Medical School, USA; 3) Preventive Medicine & Epi, Loyola Univ. Medical Center, USA; 4) Broad Institute of Harvard and MIT, Cambridge, MA.

The ability to detect recent natural selection in the human population would have profound implications for the study of human diseases. Here, we report a genome wide survey of recent selection by examining the long range haplotypes in two African-origin cohorts: 768 Nigerians and 703 African-Americans from Maywood, USA. All the DNA samples were genotyped with the Affymetrix 6.0 chip, with 857551 and 793950 SNPs passing quality control for Maywood and Nigerian samples, respectively. To reduce the computational burden, we partitioned chromosomes into blocks consisting of no more than 1500 SNPs, with each gene contained in one block. We inferred the haplotypes of each individual-with the software fastPhase. We next applied the software Sweep to detect selection signals across the genome. Similar to the previous reports in the literature, we observed widespread selection signals in the genome in both Maywood and Nigerian samples. Using the criteria that haplotype (consisting derived alleles) frequency 0.5, |iHs|2.5 and at least 3 SNPs in each core haplotype, we identified 2283 and 2072 regions that may show selection evidence in Nigerian and Maywood samples. There are 664 core haplotypes shared in the Maywood and Nigeria cohorts. Known selection evidence in genes such as CDH12, CD36 and Large is also confirmed in our dataset. Although the partially overlapping results may suggest many regions may be under population specific selection pressure due to the adaptation of environments, it may also indicate the challenge in separating true selection signals from noise using current statistical methods. Finally, the analysis in an African-American population could be confounded by admixture of chromosomes from different ancestral populations. We are testing this hypothesis by simulation of African-American chromosomes in genome regions where no selection evidence has been identified, as the null distribution.

Further Delineation of the Xp11.23-p11.22 Microduplication Syndrome. *P. Hurtado[1,2], S. Parkash[1], L. Dupuis[1], D. J. Stavropoulos[1,3], R. Mendoza-Londono[1]* 1) Div. Clinical and Metabolic Genetics, The Hospital for Sick Children, Toronto, ON, Canada; 2) Inst de Genetica Humana, Pontificia Univ Javeriana, Bogota, Colombia; 3) Dept. Paediatric Laboratory Medicine, The Hospital for Sick Children, Toronto, ON, Canada.

Pure duplications of segments of the X chromosome are rare events. Microduplications of chromosome X have been found in males with mental retardation and may involve known X-linked mental retardation genes as well as the *MECP2* gene. Females are less frequently affected possibly due to preferential inactivation of the duplicated X. We describe a 27-month-old girl who presented with status epilepticus, hypotonia, significant developmental delay and dysmorphic features including brachycephaly, triangular-shaped face with flat profile, small mouth with thin lips and small palpebral fissures. Her weight and length plotted along the 10th to 25th centile and her head circumference was on the 2nd centile. G-banding Karyotype revealed normal female complement 46,XX. Array comparative genomic hybridization (aCGH) revealed a *de-novo* duplication of ~4Mb of chromosome region Xp11.23-p11.22 involving 6 BAC clones. The duplication was confirmed by FISH with BAC clone RP11-576P23. Parental FISH analysis was normal. X inactivation studies are underway. This genomic region harbors over 20 genes. Our patient constitutes the 4th reported case of microduplication of Xp11.23-p11.22. The presence of seizures in her may be related to functional dysomy for one of the genes in this region. We present a detailed clinical comparison of the reported patients and further delineate the clinical phenotype for this newly recognized microduplication syndrome.

Specific Pur alpha-interacting proteins modulate the neuronal toxicity caused by fragile X premutation rCGG repeats. *W. Li, A. Qurashi, Y. Qin, L. Ray, P. Jin* Human Genetics, Emory University School of Medicine, Atlanta, GA.

Fragile X-associated tremor/ataxia syndrome (FXTAS) is a neurodegenerative disorder in fragile X premutation carriers with FMR1 alleles containing 55-200 CGG repeats. Using a Drosophila model of FXTAS, we previously demonstrated that transcribed premutation repeats alone are sufficient to cause neurodegeneration, suggesting that rCGG repeat-binding proteins (RBPs) may be sequestered from their normal function by rCGG binding. Recently we identified Pur alpha and hnRNP A2/B1 as RBPs, which could modulate the rCGG-mediated neuronal toxicity. To further investigate the role of Pur alpha in rCGG-mediated neurodegeneration, we have taken a proteomic approach to identify the proteins that interact with Pur alpha. Around 100 proteins, including several known interactors such as Fmrp, were found to directly interact with Pur alpha in vitro. To evaluate the potential contribution of Pur alpha-interacting proteins to rCGG-mediated toxicity, we further tested their genetic interactions with rCGG repeats using our FXTAS transgenic fly models. Interestingly, only two proteins, Rm62 and Hts, could modulate the rCGG-mediated toxicity. These data suggest that specific pathway regulated by Pur alpha could contribute to rCGG-mediated toxicity.

Evolutionary analysis across the HRAS1 minisatellite locus and its functional implication. *C. Ouyang[1], G. X. Zhang[1], S. F. Ding[1], D. D. Smith[2], T. G. Krontiris[1]* 1) Division of Molecular Medicine, Beckman Research Institute of the City of Hope, Duarte, CA; 2) Division of Information Sciences, Beckman Research Institute of the City of Hope, Duarte, CA.

We investigated a 9-kb block of sequences in strong linkage disequilibrium on chromosome 11p15 encompassing the human HRAS1 oncogene and the downstream polymorphic minisatellite (VNTR) implicated in cancer predisposition. We resequenced 12 chimpanzees and 125 individuals across three major human populations (African, European, and Japanese), identified single nucleotide polymorphisms (SNPs), genotyped VNTR alleles, and constructed full SNP-VNTR haplotypes for evolutionary analysis. Phylogenetic analysis revealed that, despite a large number of minisatellite alleles, the common haplotypes could be clustered into two major clades arising since the divergence of the human and chimpanzee lineages. The four most commonly observed European minisatellite alleles and their derivatives marked the sub-clades. SNPs tagging such haplotype lineages showed significant association with expression levels of other genes, suggesting some novel functional property of the VNTR locus. Also, population genetic analysis suggested evolutionary selection further downstream from the HRAS1 gene and the minisatellite a region where no gene was annotated, but which demonstrated significant conservation across vertebrates.

Interstitial Cystitis is a genetically heterogeneous disorder. *S. Boyden[1,3], J. Dimitrakov[2], L. Kunkel[1,3]* 1) Dept Genomics, Children's Hosp, Boston, MA; 2) Dept Urology, Children's Hosp, Boston, MA; 3) Dept Genetics, Harvard Medical School, Boston, MA.

Interstitial Cystitis (IC) is a debilitating bladder disease characterized by chronic severe pelvic pain and extreme frequency and urgency of urination. Its etiology is unknown but there is a 17-fold increased relative risk to first degree relatives of affected patients. We ascertained a group of large kindreds from Bulgaria that show autosomal dominant inheritance of IC. Members of six of these families were genotyped using a 10,000 SNP microarray and parametric linkage analysis was used to map the locations in separate families of four distinct loci that each potentially harbor a mutation that causes IC. We provide the first evidence that IC is a genetic disease that can show Mendelian inheritance, and we demonstrate that it exhibits marked locus heterogeneity. The future identification of multiple IC genes may define a biological pathway essential for proper bladder function and lead to new avenues of IC research and targeted therapeutic strategies.

The Grancalcin gene on chromosome 2q22 is a novel positional candidate for pre-eclampsia susceptibility. *E. K. Moses[1], M. P. Johnson[1], L. F. Tømmerdal[2], C. E. East[3], T. D. Dyer[1], J. Blangero[1], R. Austgulen[2], S. P. Brennecke[3]* 1) Dept Genetics, Southwest Foundation for Biomedical Research, San Antonio, Texas; 2) Department of Cancer Research and Molecular Medicine, Faculty of Medicine, Norwegian University of Science and Technology (NTNU), Trondheim, Norway; 3) Department of Obstetrics & Gynaecology, University of Melbourne, Carlton, Australia.

We are currently dissecting a susceptibility locus for pre-eclampsia on chromosome 2q22 in Australian and New Zealand families. Our strategy involves SNPing across this region with known gene-centric SNPs combined with genome wide transcriptional profiling in decidua from pre-eclamptic and control pregnancies to prioritize genes for deep re-sequencing in our affected families. We initially genotyped 586 known SNPs from within 72 genes; 414 of these SNPs were polymorphic and our preliminary statistical analysis identified only 1 SNP (rs12693126) in the proximal promoter of the *ARL5A* gene showing significant evidence of association with pre-eclampsia. We have now re-sequenced 13 genes in 48 pre-eclamptic women from our families. A total of 423 polymorphisms (414 SNPs and 9 insertion/deletion variants) have been identified, including 42 of the 586 known SNPs that we had genotyped previously. Of the 372 newly identified SNPs, 192 have now been genotyped in the original set of 34 pre-eclamptic families (The 34 Family Cohort) plus an extension of this family cohort that incorporates an additional 40 pre-eclamptic families (The 74 Family Cohort). The *ARL5A* SNP (rs12693126) showing significant association in the 34 Family Cohort ($p = 1.1 \times 10^{-4}$) was not significant in the extended 74 Family Cohort (p > 10-2). The strongest association observed ($p = 1.33 \times 10^{-5}$ in the 74 Family Cohort) was for the rs17783344 SNP resulting in a non-synonymous amino acid change [S>A] in exon 3 of the GCA (Grancalcin) gene that has not previously been implicated in pre-eclampsia. We are currently performing a replication association study with the *ARL5A* and *GCA* SNPs in a large Norwegian case and control sample derived from the HUNT population study.

Autosomal recessive woolly hair with hypotrichosis caused by a novel homozygous mutation in the P2RY5 gene.
Y. Shimomura, M. Garzon, A. Christiano Dermatology, Columbia University, New York, NY.

During the last decade, several causative genes for hereditary hair diseases have been identified, which have disclosed the molecular mechanisms involved in the hair follicle morphogenesis and cycling. We and others have recently reported that mutations in the P2RY5 gene, encoding an orphan G protein-coupled receptor, underlie autosomal recessive woolly hair and/or hypotrichosis. Although these findings clearly reveal the involvement of P2RY5 mutations in hereditary hair diseases, the clinical manifestations of P2RY5 mutations have not completely been elucidated due to limited information to date. In this study, we ascertained a consanguineous family of Iranian origin with an affected girl showing sparse and hypopigmented scalp hair. She showed a woolly hair phenotype with normal hair density at birth, but has progressed with age to show hypotrichosis. Direct sequencing analysis resulted in the identification of a novel homozygous mutation (436G>A) in the P2RY5 gene of the patient, which results in a non-conservative amino acid change (G146R) at the protein level. Our findings extend the mutation spectrum of P2RY5 mutations, and further support a crucial role of P2Y5 in hair growth in humans.

Novel de novo genomic imbalances constitute a significant fraction in a cohort of 2333 consecutive clinical samples tested by whole-genome array CGH. *S. Aradhya, S. Warren, N. Flores, J. Hilburn, C. Ding, T. Bonaga, R. Busin, G. Richard* Clinical Microarray Services, GeneDx, Gaithersburg, MD 20877.

In 2333 consecutive clinical cases referred for whole-genome oligonucleotide array CGH testing, we identified 420 (~18%) positive cases and 158 (~6.8%) cases of variants of unknown clinical significance. Many of the positive cases involved rearrangements in regions of high genomic complexity featuring a rich concentration of segmental duplications. We found such rearrangements in 1q21, 2p15, 8p23.1, 9q34, 16p11, 16p13, and 17q21, and all of these loci were recently linked to disease-causing syndromes. As expected, both deletions and reciprocal duplications were identified at many of these loci, but not all rearrangements were pathogenic. Cases classified as variants of unknown clinical significance, in contrast, were generally not found in regions of high complexity, and sometimes involved only a few genes. However, many of these variants were likely causative of clinical phenotypes-a point emphasized by the finding that a significant number of them were de novo imbalances. Half of all cases classified as positive or variant of unknown clinical significance had parental testing data. De novo genomic imbalances accounted for 102 (43%) of these cases tested for inheritance. More than half (58%) of the de novo cases were genomic imbalances that were not described previously. Of the remaining (familial) cases, most were in the variants of unknown significance category. If the cases that did not have parental testing results were to also demonstrate a similar pattern of inheritance, the rate of de novo findings alone for all cases referred for array CGH testing could approach 10%. Our findings highlight the sensitivity of a whole-genome array CGH approach and demonstrate its power to discover genomic imbalances underlying novel clinical conditions.

***TGFBR2* Mutations Reduce Expression of Contractile Proteins in Aortic Smooth Muscle Cells and Predispose Individuals to Thoracic Aortic Aneurysms and Dissections.** *C. S. Kwartler[1], S. Inamoto[1], A. L. Lafont[1], Y. Y. Liang[2], v. Tran-Fadulu[1], M. Willing[3], A. Estrera[4], H. Safi[4], M. C. Hannibal[5], J. Carey[6], J. Wiktorowicz[7], F. Tan[1], X. H. Feng[2], H. Pannu[1], D. M. Milewicz[1]* 1) Department of Internal Medicine, University of Texas Medical School, Houston, TX; 2) Department of Surgery, Baylor College of Medicine, Houston, TX; 3) Department of Pediatrics, University of Iowa, Iowa City, IA; 4) Department of Cardiothoracic and Vascular Surgery, University of Texas Medical School, Houston, TX; 5) Department of Pediatrics, University of Washington School of Medicine, Seattle, WA; 6) Department of Pediatrics, University of Utah College of Medicine, Salt Lake City, UT; 7) Department of Biochemistry and Molecular Biology, University of Texas Medical Branch, Galveston, TX.

Mutations in the TGF- receptor type II gene (*TGFBR2*) cause thoracic aortic aneurysms and dissections (TAAD). Studies have suggested a gain of function for these mutations, with increased TGF- signaling resulting in vascular disease. We characterized the phenotype of smooth muscle cells (SMCs) with *TGFBR2* mutations, and our data suggest that *TGFBR2* mutations cause a loss of function resulting in defective SMC differentiation. Primary aortic SMCs from patients (n=4) showed a global decrease in SMC contractile proteins (ACTA2, MYH11, CNN1, SMTN; $p<0.001$) by quantitative PCR and showed no change in cytoskeletal protein expression when compared with control SMCs. Patient SMCs showed increased expression of S100A4, a marker of de-differentiated SMCs ($p<0.001$). Analysis of aortas from patients (n=3) confirmed decreased expression of SMC contractile proteins. Addition of TGF- increased the expression of SMC contractile proteins in control but not mutant SMCs. Fibroblasts from patients (n=8) failed to transform into myofibroblasts on TGF- stimulation. Finally, introduction of *TGFBR2* mutations into a mouse mesenchymal cell line (10T1/2 cells) disrupted expression of contractile proteins. These data suggest that *TGFBR2* mutations disrupt differentiation of SMCs and myofibroblasts. This is the first genetic defect identified to lead to defective SMC differentiation.

Genomewide copy number analysis in Cornelia de Lange Syndrome. *M. Deardorff[1], M. Kaur[1], M. Berman[1], L. Conlin[1], D. Clark[1], X. Gai[2], J. Perin[2], T. Shaikh[1], H. Hakonarson[1,3], L. Jackson[4], I. Krantz[1]* 1) Division of Genetics; 2) Bioformatics Department; 3) Center for Applied Genomics, Children's Hospital of Philadlephia, PA; 4) Drexel School of Medicine, Philadelphia, PA.

The Cornelia de Lange syndrome (CdLS) is a multisystem developmental disorder with facial dysmorphia, growth and cognitive retardation, gastrointestinal abnormalities and limb deficiencies. We have identified mutations in 65% of patients with CdLS. These mutations involve the genes *NIPBL*, *SMC1A* and *SMC3*, all of which are involved in sister chromatid cohesion, a process that regulates proper segregation of sister chromatids. We have yet to elucidate a cause of CdLS in 35% of patients and to facilitate identification of genes in CdLS we have used genome-wide CNV analysis. To date, 269 individuals submitted to our study for whom causative mutations had not been previously identified were analyzed using Illumina HapMap550K SNP arrays.

Analysis revealed 8932 potential variants ranging from 2bp to 20Mb with 5191 being 5 SNPs or less. Computational and manual curation prioritized potential causative variants that could contain candidate CdLS genes. We have identified a number of large chromosomal abnormalities in individuals with phenotypic overlap with CdLS, but who did not meet full clinical criteria. We also identified deletions in 5 patients that included *NIPBL* and were validated using MLPA. There were no deletions that included *SMC1A* and *SMC3*, consistent with previous findings of only missense mutations in these genes. We have identified CNVs involving several additional genes and are in the process of validating these abnormalities. We are currently using the same array platform to analyze an additional 250 unaffected parents, to facilitate the identification of de novo CNVs, and 150 mutation-positive patients, to quantify whether there is previously unappreciated aneuploidy in patients with CdLS. This work has provided potential leads for additional candidates for CdLS and is leading to increased insight of the molecular basis of this multisystem disorder and overlapping phenotypes.

Fragile X Mental Retardation Protein Interactomes in Drosophila and Mouse. *L. Lin, Y. Qin, L. Ray, P. Jin* Human Genetics, Emory University School of Medicine, Atlanta, GA.

Fragile X syndrome is caused by the functional loss of fragile X mental retardation protein (FMRP). FMRP is a selective RNA-binding protein that forms a messenger ribonucleoprotein (mRNP) complex associating with polyribosomes. FMRP, as an RNA-binding protein, has been implicated in mRNA transport and translational control. To further understand the biological functions of FMRP, we have taken a proteomic approach to identify the proteins that interact with FMRP. Both fly and mouse GST-Fmrp fusion proteins were used to capture the brain proteins directly interacting with Fmrp. Over one hundred distinct proteins were captured with either fly or mouse GST-Fmrp. Several known Fmrp interactors, such as FXR1, FXR2 and nucleolin, were captured. Importantly both fly and mouse orthologs of several novel proteins were also identified, including Nono, Heat shock protein 8 and GPI-anchored membrane protein 1. Some of them displayed the genetic interaction with Fmrp in our gain-of-function fly model as well, suggesting that they play a role in the pathways regulated by Fmrp. This systematic approach to identify the Fmrp-interacting proteins in both fly and mouse will facilitate our further understanding the biological roles of FMRP.

Clinical characterization of two siblings with microdeletion 15q13.2q13.3 identified through chromosomal microarray analysis. *J. Rousseau[1], S. Ben-Shachar[2], T. Sahoo[2], F. Dibazar[1], B. Moghaddam[1]* 1) Section of Genetics, Department of Pediatrics, UC Davis Medical Center, Sacramento, CA; 2) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Microdeletion of 15q13.3 is a newly emerging syndrome of mental retardation, seizures, and subtle dysmorphic features. We report here two siblings who presented for genetics evaluations due to their personal and family history of mental retardation and were subsequently found to have a microdeletion of 15q13.3 through chromosomal microarray analysis (CMA). Both children have very subtle dysmorphic facial features and no congenital anomalies; therefore, we suggest that CMA is warranted even in the absence of such findings. Patient 1 is a 12-year-old female and patient 2 is her 9-year-old full sister. The sisters' biological mother is reported to have mild mental retardation and her biological father to have unknown psychiatric issues and probable cognitive impairment. Both patient 1 and patient 2 have been diagnosed with mild mental retardation. On physical examination, both are noted to be of average stature with normal weight and head circumference. Their subtle dysmorphic features include a narrow and elongated face with a pointed jaw, mild hypertelorism, a high arched palate, and elongated digits. Both are also noted to be hyperextensible and hypotonic. Patient 2 also has cupped ears, wide teeth with an overbite, and thoracic scoliosis. Characterization of the deleted region in both sisters defined its size to be ~1.6Mb. Parental studies are not possible due to the social situation. CHRNA7 is responsible for neuronal nicotinic cholinergic signal transmission mediation and has been associated with epilepsy and schizophrenia. Identifying deletions of this gene may affect medication choices in patients diagnosed with microdeletions of 15q13. Our two patients will undergo formal neurodevelopmental and psychiatric evaluations. We present these sisters as two patients who have a newly emerging syndrome associated with a microdeletion of 15q13 and to illustrate that such deletions may be identified in patients who have only very subtle dysmorphic features and developmental delay.

Estimating pleiotropy in pigmentation traits using admixed populations. *E. E. Quillen[1], S. Beleza[2], R. A. Kittles[3], R. W. Periera[4], J. Rocha[3], T. Frudakis[5], M. D. Shriver[1]* 1) Department of Anthropology, Pennsylvania State University, University Park, PA; 2) IPATIMUP, University of Porto; 3) Department of Medicine, University of Chicago; 4) Department of Genomics and Biotechnology, Catholic University of Brasilia; 5) DNAPrint Genomics, Inc., Sarasota, FL.

There are obvious correlations among pigmentation traits across populations. Individuals from West Africa tend to have darker skin, hair, and eyes relative to Northern Europeans. To determine if these correlations are due to population stratification or pleiotropy, this study examined two admixed populations, African Americans and Brazilians, for whom biogeographic ancestry (BGA) was calculated using MLE on 176 ancestry informative markers (AIMs) and for whom iris, skin, and hair pigmentation values were determined. Because these traits are correlated with BGA, as well as with one another, it is essential to remove this confounding effect before estimating the correlation among the phenotypes. For each population, the phenotype values were transformed using the regression equation for the phenotype and BGA for that population. The correlations between the transformed pigmentation values then yield a more accurate estimate of the degree of pleiotropy that is expected for each set of phenotypes in each population. In all cases, the correlations between phenotypes were reduced when the effect of their correlations with BGA were eliminated. Significant correlations remained in the African Americans for skin and iris color and in the Brazilians for skin and hair color. Genetic variation resulting in phenotypic variation in multiple pigmentation traits is expected since melanin production and distribution is essential to all three of these phenotypes. *KITLG* is an attractive candidate gene for a pleiotropic effect because it has been previously associated with both skin and hair color. These results suggest that while there is likely to be pleiotropy affecting pigmentation phenotypes, a substantial amount of variation may be due to genes affecting only one of the phenotypes. This appears to be the case for *TYRP1* which influences iris pigmentation in the African American sample but has no effect on skin or hair pigmentation.

Characterization of a Novel MeCP2 Isoform and its Role in Rett Syndrome. *P. J. Gianakopoulos, A. Mikhailov, B. Stachowiak, J. B. Vincent* Neurogenetics, Centre for Addiction and Mental Health, Toronto, Canada, Canada, M5T 1R8.

Rett syndrome (RTT) is a postnatal neurodevelopmental disorder that primarily affects females at a frequency of 1:10 000 births. Following a period of seemingly normal development of 6-18 months, a baby will rapidly regress, demonstrated by a loss of motor skills and communication; and autistic-like social behaviour, ultimately acquiring severe mental retardation. In 1999 it was discovered that mutations in MeCP2 were responsible for RTT. MeCP2 binds to methylated CpG dinucleotides and has been shown to be a regulator of transcription via its interaction with chromatin-modifying factors. In the known transcript of the Mecp2 gene, termed MeCP2_e2, all four exons are utilized. We recently identified a new variant of the MECP2 gene that is the result of alternative splicing, whereby the second exon is spliced out. There is strong evidence that this variant, termed MeCP2_e1, may be the etiologically relevant form in RTT. The main objective of this study is to delineate the relevant splice variant(s) of the Mecp2 gene that contributes to the neuropathology of Rett Syndrome. To undertake this stable clones of COS-7 cells were produced expressing either of the two splice variants of MeCP2 along with appropriate controls. Total RNA was extracted from 4 clones of each cell line and gene expression microarray was carried out as a service by the University Health Network Microarray Centre (Toronto, Canada) using Agilent rhesus monkey oligonucleotide arrays. Results indicated that MeCP2_e1 and MeCP2_e2 differentially regulated, by at least 2-fold, 71 and 115 genes, respectively, whereas 82 genes were targeted by both MeCP2 isoforms. Several neuronal-specific genes and histone gene family members were identified as genetic targets of one or both MeCP2 splice variants underscoring their function in neuronal modelling and chromatin remodeling. Understanding the differences between the two MeCP2 isoforms will have important implications, particularly for understanding the neurobiology of the disease, and for the development of future treatments for Rett.

Association of the Caucasian haplotype AH8.1 with AIDS-related Non-Hodgkin's Lymphoma. *B. Aissani[1], M. O. Kisani[2], S. Shrestha[1], J. Tang[2], L. P. Jacobson[3], E. Breen[4], O. Martinez-Maza[5], R. A. Kaslow[1,2]* 1) Epidemiology, UAB, Birmingham, AL; 2) Medicine, UAB, Birmingham, AL; 3) Epidemiology, Johns Hopkins University, Baltimore, MD; 4) Psychiatry, UCLA, CA; 5) Obstetrics-Gynecology, UCLA, CA.

OBJECTIVE: The genetic determinants of predisposition to Non-Hodgkins lymphoma (NHL) are poorly understood; however, a polymorphism in lymphotoxin alpha LTA (+252G) plus a polymorphism defining the high tumor necrosis factor (TNF)-producing allele (-308A) form the G-A haplotype that has been associated with NHL alone or in combination with HLA-B*08 or HLA-DRB1*03. Whether any of those gene variants is a true etiologic factor remains uncertain because they occur on the highly conserved Caucasian haplotype AH8.1. We aimed to determine whether any of these gene variants are also associated with susceptibility to AIDS-related NHL (AIDS NHL). METHODS. We typed 159 pairs of AIDS-NHL cases and matched controls selected from HIV-infected European American men in the Multicenter AIDS-Cohort Study for 63 SNPs across the LTA/TNF and the complement C2-C4B gene regions, and for 6 HLA class I and II genes. To account for confounding by the duration of HIV infection, we adjusted for CD4+ T-cell count at the time of diagnosis in the index cases. Logistic regression and haplotype trend regression models were used to assess the risk associated with extended HLA haplotypes. RESULTS. The G-A haplotype and alleles at 4 other SNPs in the C2-C4B region were associated with 1.5-3.0-fold increases of risk. Overall allele distributions at the HLA class I and HLA class II loci tested were not associated with AIDS-NHL. In multivariate analyses of haplotypes, only the G-A haplotype and a 4-SNP haplotype across the C2-C4B region were associated with NHL (OR=2.6, 95% CI: 1.4-4.7; OR=3.2, 95% CI: 1.5-6.9, respectively). These two haplotypes occur in combination with B*08 and DRB1*03 on the AH8.1. CONCLUSIONS. The haplotype AH8.1 confers an elevated risk for AIDS NHL as it does for non-AIDS NHL. The implication is that many variants in the numerous immune genes on the AH8.1 are as plausible etiologic determinants of AIDS NHL as the G-A haplotype.

Mapping copy-number variation in the ENCODE regions in different human populations using high resolution comparative genomic hybridization (HR-CGH). *F. Grubert[1], A. E. Urban[1,2], J. O. Korbel[3,4], D. Palejev[1], K. Kidd[1], M. Snyder[2], S. Weissman[1]* 1) Dept Genetics, Yale Sch Medicine, New Haven, CT; 2) Molecular, Cellular & Developmental Biology, Yale University, New Haven, CT; 3) Molecular Biophysics and Biochemistry Department, Yale University, New Haven, CT; 4) European Molecular Biology Laboratory, D-69117 Heidelberg, Germany.

Copy-Number Variation (CNV) and Copy-Number Polymorphisms (CNPs) are a pervasive architectural feature of the human genome and are expected to contribute significantly to phenotypic variation both in the healthy individual and in disease states. Array-CGH typically has a predictive resolution of about 50 kb. CNV below that horizon will be missed, as will be the actual breakpoint-sequence of the variant. For a complete catalog of human CNV a procedure that predicts CNV at sub-exon level is needed. In our study we use HR-CGH based on high-density oligonucleotide tiling microarrays [Urban, Korbel et al. PNAS 2006; Korbel, Urban et al. PNAS 2007]. Maskless Synthesis arrays with 385000 oligomers were constructed to represent the non-repetitive part of the 44 ENCODE regions corresponding to 1% of the entire human genome. This provides a tiling density of one 50mer every 38bp, resulting in an overlapping tiling path. The arrays are probed with fragmented full-complexity genomic DNA. The ratio of signal intensities from control and experimental channel are processed by our /BreakPtr/ algorithm, which predicts copy-number changes and breakpoints while screening out false-positive calls caused by cross-hybridization. Predictions are validated by PCR and direct sequencing of the resulting amplicons. Using this approach we have studied CNVs and CNPs, and their breakpoints, in 36 individuals of different ethnicities. Among those are 17 individuals from the HapMap panel (Caucasians, Asians, and Africans) and 19 from populations around the world (Surui, Nasioi, Masai, Mbuti, Sandawe, Yakut). We have detected, cataloged and verified numerous variations, both such that were previously known and novel variants, with the arrays allowing to detect CNV as small as 1 kb. This study adds information about the nature of CNV in different human populations.

High-resolution fluorescence in situ hybridization (FISH) using chemically synthesized oligonucleotide libraries.

P. Tsang[1], N. A. Yamada[1], R. Ach[1], L. Carr[1], N. Sampas[1], B. Peter[1], T. Dennis[2], M. Bittner[2], S. Laderman[1], L. Bruhn[1]
1) Agilent Technologies, Inc., Santa Clara, CA; 2) TGEN, Phoenix, AZ.

Fluorescence in situ hybridization (FISH) remains the preferred method for visualization of chromosomes in cytogenetics; however, developments in genomic measurements are unmasking variations that require visualization of smaller and more complex regions that challenge current FISH technology. To address the growing need to study such loci, we constructed high-complexity oligonucleotide libraries (>200 oligos per locus), consisting of chemically synthesized long oligonucleotides (166~194mers) of high fidelity (<0.4% error rate), to enable oligonucleotide-fluorescence in situ hybridization (oFISH) of complex sequences at high resolution. oFISH probes are generated by PCR amplification of the libraries, followed by fluorescent labeling. We designed our probes to defined regions of the reference genome sequence, while avoiding sequences that are repeat-rich or homologous to non-targeted areas, thereby improving specificity while eliminating the need for Cot-1 or other suppressive hybridization reagents. Routine work requires simple protocols that closely mimic current FISH procedures and is conducted using much smaller target sizes (<20 kb) than BAC-based FISH. We have successfully detected specific and robust metaphase and interphase staining at various loci on multiple chromosomes including one particularly repeat-rich region (53% repeat-masked). In addition, we achieved multi-color detection of regions flanking the centromere of the X-chromosome by creating p-arm probes in red and q-arm probes in green, positioned 624 kb and 384 kb away from the centromere, respectively. These centromere-flanking probes are free of repetitive sequences and may provide advantages over currently available centromeric probes that can cross-hybridize due to their repetitive nature. These results suggest that the resolution, specificity and flexibility afforded by oFISH meet the growing need to visualize microarray- and sequencing-based findings of fine scale structural variations and other variations throughout the genome.

Overlapping spectrum of mutations causing distal arthrogryposis type 1 and 2. *P. Kezele[1], A. E. Beck[1], M. J. McMillin[1], R. Toydemir[2], M. J. Bamshad[1]* 1) Dept Pediatrics, University of Washington, Seattle, WA; 2) Department of Human Genetics, University of Utah, Salt Lake City, UT.

The distal arthrogryposis (DA) syndromes are a group of 10 dominantly inherited disorders characterized by multiple congenital contractures such camptodactyly and clubfoot. DA syndromes are caused by mutations in at least seven different genes that encode components of the contractile apparatus of fast-twitch myofibers. We previously reported that distal arthrogryposis type 1 (DA1) is caused by mutations in *TPM2* whereas distal arthrogryposis type 2B or Sheldon-Hall syndrome (SHS) is caused by mutations in *MYH3*, *TNNI2*, or *TNNT3*. SHS has traditionally been distinguished from DA1 by the presence of contractures of the facial muscles and subtle differences in the pattern of limb contractures. However, making the distinction between the DA1 and SHS is often challenging. We screened a cohort of about 125 probands with either DA1 (n = ~50) or *MYH3*-negative SHS (n = ~75) with well-characterized phenotypes for mutations in *TNNI2*, *TNNT3*, and *TPM2*. Mutations were found in ~20% of probands, found more frequently in SHS than in DA1 and found more often in familial cases compared to sporadic cases. These results suggest that there are yet to be discovered genetic and/or environmental risk factors of DA1 and SHS. The phenotypic overlap between DA1 and SHS cases was substantial and frequently a diagnosis of SHS rather than DA1 was based on the presence of calcaneovalgus defects, deep nasolabial folds and a somewhat small mouthеach of which has also been reported at low frequency in DA1. Consistent with these observations, mutations in *TNNI2*, *TNNT3*, and *TPM2* were found in both probands with DA1 and probands with SHS. There was no consistent relationship between genotype and phenotype. Therefore, DA1 and SHS appear to represent the different tails of a distribution of phenotypic characteristics caused by mutations in *TNNI2*, *TNNT3*, and *TPM2*. Delineation of these syndromes as separate entities was critical to the process of gene discovery for DA disorders and yet further investigation now demonstrates that they share an overlapping genetic and phenotypic spectrum.

Vitamin D receptor: a versatile genetic modifier for Alzheimer and Parkinson disease. *K. Hara[1], L. Wang[1], G. Wang[1], G. Beecham[1], P. Gallins[1], P. Whitehead[1], M. Greg[1], M. Butler[1], K. Yu[1], E. Martin[1], J. Haines[2], J. Gilbert[1], J. Vance[1], M. Pericak-Vance[1]* 1) Human Genomics, Miller School of Medicine, University of Miami, Miami, FL, USA; 2) Center for Human Genetics Research, Vanderbilt University, Nashville, TN, USA.

By converging genomic evidence from published association and genome-wide linkage association, and expression studies, we identified the vitamin D receptor (*VDR*) gene as a strong candidate for late-onset Alzheimer disease (LOAD). *VDR* is a nuclear receptor for vitamin D, known to be a neuroprotective factor. We sought to thoroughly evaluated association in *VDR* using 492 cases with LOAD and 496 controls. First, we re-sequenced 96 chromosomes to catalogue all exonic and promoter SNPs(-6.0 kb) of *VDR*. The SNP panel used to evaluate the gene includes all HapMap phase II tagSNPs and all SNPs identified by re-sequencing. Analysis of the entire set of 68 *VDR* SNPs found association between 12 SNPs and AD risk. All the significant SNPs are located in the intron 1, 2, and the promoter region. The most significant associations were found at promoter SNP rs11568820 and rs7976091 ($P=9.0510^{-6}$, OR=1.69; $P=8.9410^{-5}$, OR=1.55, respectively). The two SNPs are in complete linkage disequilibrium with each other ($r^2=1$). We then conducted a family-based association analysis of the most interesting 19 SNPs and demonstrated significant allelic association for 6 SNPs among families with affected *APOE4* carriers (P=0.01 to 0.05). Given that the *VDR* gene expression was also associated with Parkinson disease (PD) risk, we evaluated association in *VDR* gene using a large PD family data set (N=3357). Strikingly, the same SNPs associated with AD risk were also associated with age-of-onset of PD, with the strongest association at rs4334089 (P=0.0008; P=0.0005 with AD risk). rs11568820 lies within a transcription factor binding site and rs4334089 resides in a conserved region with high regulatory potential. Preliminary luciferase reporter assay suggest that allelic differences at rs11568820 in the *VDR* promoter indeed affect transcriptional activity. Our data strongly indicate that *VDR* is a genetic modifier for two major neurodegerative diseases.

A framework for genomic risk counseling for common disease variants. *E. Levin, K. Kaplan, S. Kieran* Genetic Counseling Program, Navigenics, Redwood Shores, CA.

Genetic predisposition risk assessment has traditionally relied on tools like personal and family medical history, standard screening or diagnostic tests, and targeted monogenic genetic testing. While this approach is sufficient for certain well-defined hereditary conditions (e.g., cystic fibrosis, Lynch syndrome, Huntingtons Disease, etc.), genetic professionals have been limited in their ability to offer risk assessment for common, multifactorial conditions like diabetes, heart disease, and many cancers. With the discovery of single nucleotide polymorphism (SNP) associations with common diseases, new genomic tests are emerging that provide an additional, powerful tool that can be used as an adjunct to traditional risk assessment tools to inform about predisposition to common diseases. Genetic counselors and other clinical genetic professionals will be at the forefront of delivering this new service and will likely play a critical role in interpreting genomic test results and providing genomic risk counseling. However, one of the main challenges will be how to incorporate results from genome-wide assessments into traditional practice, as no guidelines currently exist. We propose a framework for providing genomic risk assessment. Core components include: discussing the benefits, risks, and limitations of genome-wide testing; explaining the effects associated with multiple, common SNPs versus rare, single gene mutations; assessing medical and family history for possibility of a highly penetrant gene mutation; evaluating patients health behaviors and lifestyle; explaining environmental contributions to disease risk; exploring patients personal experience with disease and motivations for pursuing genomic risk assessment; and reviewing strategies for disease prevention and early detection. Further, based on experience we explore common questions and reactions to genomic risk assessment to further outline the psychosocial considerations that need to be recognized and addressed. As this field evolves, this framework provides a basis for developing future professional practice guidelines.

Diagnositic testing for Smith-Lemli-Opitz syndrome by sequence analysis: a two tier approach. *S. F. Suchy, J. D. Higgs* GeneDx, Gaithersburg, MD.

Smith-Lemli-Opitz syndrome (SLOS) is an autosomal recessive disorder of cholesterol biosynthesis due to mutations in the gene for 7-dehydrocholesterol reductase (DHCR7) that catalyzes the last step in cholesterol biosynthesis. Deficiency in DHCR7 results in global psychomotor retardation, failure to thrive, and multiple congenital malformations including dysmorphic facial features, microcephaly, cleft palate, syndactyly, polydactyly, and malformations of the brain, heart, gastrointestinal tract, genitalia and kidneys. Most patients have elevated 8-dehydrocholesterol (8-DHC) and 7-dehydrocholesterol (7-DHC) levels. One of the early indications of SLOS is a low maternal serum unconjugated estriol level, used as part of the test to screen for neural tube defects, Down syndrome and trisomy 18. *DHCR7* mutation testing, DHCR7 enzyme assay or the analysis of sterol biosynthesis in cultured cells are recommended methods to confirm the diagnosis of SLOS for family studies and in rare cases where levels of 7-DHC and 8-DHC are normal or in the range of heterozygotes. According to a large study of individuals with clinically and biochemically characterized SLOS, sequence analysis identified mutations in 96% of patients (Witsch-Baumgartner et al., 2000). As most *DHCR7* mutations occur in exons 6, 7, 8 and 9, we perform *DHCR7* sequence analysis by testing these exons first (Tier 1), followed by the rest of the exons (Tier 2). Of 64 probands referred to us for molecular testing for SLOS 36 patients had 2 or more mutations identified (56%). No patients in our cohort had only a single mutation identified. Of the patients with two mutations identified 30/36 had two mutations identified in Tier 1 exons, 4 patients had at least one mutation identified in Tier 1 and one in Tier 2 and one patient had two mutations identified in Tier 2. Overall, of 74 mutations identified, 66 mutations were found in Tier 1 exons (89%) and 8 in Tier 2 exons. The most common *DHCR7* mutation IVS8-1 G>C, was detected in 32/74 alleles (43%). These findings are consistent with previous report in a research setting (Witsch-Baumgartner et al., 2000) and confirm the value of a tiered approach in molecular testing for *DHCR7* mutations in a diagnostic laboratory.

Longitudinal genome-wide association of cardiovascular disease risk factors in The Bogalusa Heart Study. *E. Smith[1], M. Shaw[1], R. Salem[2], S. Srinivasan[3], W. Chen[3], E. J. Topol[1], N. J. Schork[1], G. S. Berenson[3], S. S. Murray[1]* 1) Scripps Genomic Medicine, Scripps Translational Science Institute, La Jolla, CA; 2) University of California, San Diego, CA; 3) Tulane University, New Orleans, LA.

Identifying the genes that contribute to CVD risk profiles will lead to direct insights not only into the mechanisms that the genes work through to cause CVD, but also insights into the clinical profiles that signal genetically-mediated susceptibility and early onset of disease. Previous genome-wide association (GWA) studies have found numerous associations with CVD and CVD risk factors in cross-sectional data. We are currently performing a GWA study on CVD risk factors using longitudinal data from >1200 individuals in the Bogalusa Heart Study (BHS), a long-term, epidemiologic program in a biracial community (35% African-American (AA) and 65% European-American (EA)). The BHS began in 1973 and 7 cross-sectional CVD risk factors screenings of children aged 5 to 17 years were conducted between 1973 and 1992. In addition, 8 cross-sectional screenings of young adults who had been previously examined as children have been conducted to date. We have genotyped these individuals at >650,000 SNPs and copy-number variable regions using Illuminas Human610-Quad and HumanCVDv2.0 genotyping BeadChips. In the Human610 BeadChip, we have observed a 99.95% call rate from 590,622 loci and 1234 samples, and >99.99% reproducibility in 34 replicate DNA samples. We have examined loci previously described in cross-sectional data in both EA and AA populations using mixed effect regression models with patterned covariance matrices, accounting for age and gender. In the EA population, many previously described associations were replicated, including 1p13.3, which was associated with LDL levels ($p = 8 \times 10\text{-}5$) as well as with changes in LDL over time ($p = 0.001$). We are currently analyzing all genome-wide SNP data and exploring analyses addressing interactions between specific markers and genetic background, copy number variations, and multi-trait models. Overall, our GWA study on longitudinal data provides insight into the genetic basis of how individuals develop disease over time, from childhood into adulthood.

Haplotype-based Linkage of *ZNF699* to Alcohol Dependence and Alcohol Response in *LaJolla* Population. *J. Liu[1], Z. Zhou[1], M. Schuckit[2], T. Smith[2], C. Hodgkinson[1], P. Shen[1], D. Goldman[1]* 1) Laboratory of Neurogenetics, National Institute on Alcohol Abuse and Alcoholism, Rockville, MD; 2) Alcohol Research Center, VA San Diego Healthcare System, La Jolla, CA 92093-116A.

Development of tolerance is important in the progression to Alcohol Dependence (AD). Ethanol tolerance in Drosophila depends on two distinct molecular pathways: the octopamine pathway and a newly discovered cellular stress pathway within which functions hangover, a gene encoding a large nuclear zinc-finger protein. Human ZNF699 (Chr.19p13.2) is annotated in the NCBI database as a hangover ortholog. Recently, Riley et al associated ZNF699 to Alcohol Dependence in the Irish Affected Sib Pair Study, finding strong evidence for single marker association with AD, and a highly significant haplotypic association. To test the ZNF699 gene and human alcohol response and Alcohol Dependence we evaluated four SNPs that had individually shown significant association to Alcohol Dependence and two other SNPs located in the same haplotype block in LaJolla Alcohol Response dataset. We found no single marker association. But we did find that haplotype 211111 significantly associated with Alcohol Dependence (p=0.0004), with a frequency of 0.19 in cases and 0.02 in controls. The copy number of haplotype 211111 was significantly associated with alcohol abuse (p=0.0018) , but show no correlation between haplotype 211111 and level of response (Zpeakmin) and MaxDrink across four time points in LaJolla population. Our results implicate ZNF699 in alcohol response, but these findings need to be amplified in a larger sample for confirmation.

Obsessive Compulsive Disorder Collaborative Genetics Study (OCGS) candidate gene analysis: Association of *SLC6A4* variants with OCD in a large multi-center US family study. *E. Voyiaziakis on behalf of the OCD Collaborative Genetics Study (Brown U, Columbia U, Harvard U, JHU, NIMH & UCLA)* USC, Keck School of Med., Psychiatry & Behavioral Science, Los Angeles, CA.

Studies that have investigated a genetic association between *SLC6A4* and OCD have been equivocal. To resolve ambiguity in association findings, we genotyped the *OCGS* sample of 1241 persons from 278 pedigrees for 13 SNPs (ABI SNPlex), the LPR indel and molecular haplotypes of the LPR (*MSP1* endonuclease digest), two VNTR polymorphisms in introns 2 (Int2) and 7 (Int7), and a 381-base pair deletion 3 to the LPR. These data were then analyzed using FBAT with additive, dominant and genotypic models, using both OCD and sex-stratified OCD as the phenotype.

2-point FBAT analysis using a genotypic model detected nominal association with **Int2** (p= 0.0089) and **Int7** (p= 0.0187; negatively associated). All haplotypes including these polymorphisms gave significant corrected whole marker minimal p-values 0.05: **rs140700:Int7:rs4583306**, p= 0.0290; **Int7:rs4583306:rs7224199**, p= 0.0345; and **Int2:rs140700:Int7**, p= 0.0483. A sex analysis using FBAT revealed sexual dimorphism not stipulated previously. 2-point analysis showed strong association in females with **Int2** (p= 0.00018), significant after correction for LD, multiple marker- and model testing (p_{Adj}= 0.0069). The most significant haplotypes in females included: **rs140700:Int7:rs4583306**, p= 0.0133, and **Int7:rs4583306:rs7224199**, p= 0.0193.

We tested several hypotheses regarding the LPR indel. We did not observe over-transmission of the $\mathbf{L_A}$ allele as reported by Hu et al (2006). Likewise, we argue against the rare **425V** variant causing OCD, i.e., the OCD plus syndrome (Ozaki et al, 2003), as this variant did not segregate with OCD. In conclusion, we confirmed association at the *SLC6A4* locus with OCD by 2-point and haplotype analysis. However, current hypotheses regarding involvement of specific variants in conferring risk for OCD must be refined to accommodate the divergent data, including possibly differential gender risk.

Power of deep all-exon resequencing for discovery of human trait genes. *G. Kryukov[1], A. Shpunt[1,2], J. Stamatoyannopoulos[3], S. Sunyaev[1]* 1) Division of Genetics, Brigham & Womens Hospital and Harvard Medical Sch, Boston, MA; 2) Department of Physics, Massachusetts Institute of Technology, 77 Massachusetts Ave, Cambridge, MA 02139; 3) Department of Genome Sciences, University of Washington, 1705 NE Pacific Street, Seattle, WA 98195.

Availability of large genotyping microarrays propelled Genome-Wide Association Studies as a major tool for genetic analysis of complex diseases. Development of low cost sequencing technology is a widely anticipated next technological breakthrough, which is paralleled by the initiatives to phenotype hundreds of thousands of individuals. We investigate the potential of these two technological advances to enable fundamentally new ways for identification of genes underlying human complex phenotypes. While knowledge of all variants segregating in the population and identified by sequencing would seem to increase the power of genetic analysis, this prospect faces daunting statistical challenges, since an expanding pool of variants requires more stringent multiple testing correction whereas the power to detect association with low-frequency variants is reduced. Thus, we analyzed the potential of the unbiased gene discovery strategy that combines multiple rare variants from the same gene and treats genes rather than individual alleles as the unit for the association test. Our study was based on a population genetic model inferred from the deepest systematic re-sequencing dataset to date. The model incorporates incoming mutations, demographic history of the human population and natural selection. We demonstrate that the model is able to accurately recapitulate a large experimental dataset. We use this model to simulate genetic variation in larger population cohorts. We further simulate sequencing of samples from phenotypic extremes to evaluate power of unbiased discovery of genes underlying the phenotype. Our results suggest that genome-wide analysis of rare coding variation in individuals at phenotypic extremes will provide a powerful tool for discovery of new gene-phenotype associations. However, these studies would require sequencing of very large population panels exceeding 10,000 individuals.

Summary After-Segmentation Algorithm (SASA) for the analysis of copy-number variation. *C. Rangel-Escareño, V. Espinosa-Mateos, A. Hidalgo-Miranda, L. Uribe-Figueroa, R. Goya-Ogarrio, S. March, G. Jimenez-Sanchez*
National Institute of Genomic Medicine, Mexico.

High-density oligonucleotide arrays such as Affymetrix 500K and SNP6.0 allow the analysis of copy number variation (CNV) with an unprecedented resolution. This represents an opportunity for the detection of specific genes related with human diseases but nevertheless a considerable bioinformatic challenge. This task has been particularly difficult given the large amount of data generated. Current tools for this analysis include GISTIC (Rameen Beroukhim et al. 2007) and Partek (www.partek.com/). We present a new algorithm (SASA) implemented in R for detecting common altered regions and producing a graphic summary of the results after initial processing with aroma.affymetrix R-package available in Bioconductor (Henrik Bengtsson 2008). SASA searches for the critical regions throughout samples data and generates a list of regions and their corresponding frequencies based on a threshold. The highest frequency corresponding to the most common region. In addition, it graphs the frequencies and amplitude of such aberrations. To test the application of SASA we analyzed Affymetrix 500k and SNP6.0 array data from 3 normal and 15 cancer cell lines. Data were pre-processed using three different normalization algorithms and two different segmentation algorithms to also assess performance of different combinations of normalization and segmentation algorithms. Results included known regions with CNVs, including a wide amplification of chromosome 5p15 in HeLa and other 7 cell lines including the C33A for uterine cervix, the colon COLO205, and breast HS578T. 72% of the cell lines showed amplification in chromosome 2p11.2 and 44% of them coincide in amplification in 2p24.3 including HCT15 and COLO205. The SASA algorithm detects critical regions and aberration amplitudes in a timely manner facilitating analysis of CNVs.

A Recurrent Point Mutation and a Founder Copy Number Variant in SEPT9 Explain a Large Fraction of Hereditary Neuralgic Amyotrophy. *M. C. Hannibal[1], M. L. Landsverk[1], E. K. Ruzzo[1], H. C. Mefford[2,3], K. Buysse[4], J. G. Buchan[1], S. M. Seanez[1], D. M. Knutzen[1], L. R. Miller[1], J. R. Adkins[1], E. E. Eichler[3], P. F. Chance[1]* 1) Dept. of Pediatrics; 2) Dept. of Internal Medicine; 3) Dept. of Genome Sciences, University of Washington, Seattle, WA; 4) Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium.

Hereditary neuralgic amyotrophy (HNA) is an autosomal dominant syndrome that features painful episodic motor and sensory attacks and, in most cases, characteristic facial features. Relative hypotelorism is common, and occasional cleft palate is seen in individuals with HNA. The focal, recurrent peripheral neuropathy primarily affects the brachial plexus, but other nerves may be involved. Previously, we identified HNA point mutations in the SEPT9 gene encoding septin-9. Here, we report the characterization of HNA-causative mutations by sequence analysis and detection of copy number variants in the region of SEPT9. We identified exonic point mutations in 22% (11/49) of our HNA pedigrees. Interestingly, 9/11 have a recurrent NM_006640.4:c.262C>T mutation at a hypermutable base in a CpG island. Two HNA pedigrees have a NM_006640.4:c.278C>T mutation. Another 24% of our HNA pedigrees have a shared founder haplotype that was identified among North American pedigrees of European descent. We have now identified an intragenic genomic duplication of approximately 38 kilobases in these pedigrees. We find several other pedigrees have heterogeneous copy number variants within this region. SEPT9 transcription and translation is complex. Alternative promoter and 5 exon usage leads to multiple SEPT9 transcript variants encoding at least five unique protein isoforms. The founder genomic duplication results in SEPT9 transcripts that contain an in-frame, tandem duplication of the 645 bp exon containing the HNA point mutations. Immunoblotting demonstrates that this duplication results in expression of several SEPT9 protein isoforms of increased molecular weight. The duplicated 645 bp exon encodes most of the N-terminal proline-rich domain of the SEPT9, suggesting that it is important for SEPT9 function in morphogenesis and peripheral nerve maintenance.

Variants in the Human Trehalase Gene (TREH) Determine Trehalase Activity and are Associated with Type 2 Diabetes. *Y. Muller, R. Hanson, G. Nishimoto, J. Loebel, S. Kobes, J. Goswami, W. Knowler, C. Bogardus, L. Baier*
Epidemiology & Clinical Res, NIDDK/NIH, Phoenix, AZ.

The disaccharide trehalose, found primarily in insects and mushrooms, is hydrolyzed into 2 molecules of glucose by the enzyme trehalase. Deficiency of trehalase activity leads to trehalose intolerance which occurs in 15% of Greenland Eskimos. We have identified linkage to plasma/serum trehalase activity (LOD=7.0) in 570 non-diabetic Pima Indians at the trehalase locus TREH on Chr11q23. This same region was previously linked to body mass index (BMI; LOD=3.6) and type 2 diabetes (T2D; LOD=1.7) in 1037 Pima Indians. To identify the genetic variant(s) that control TREH activity, the coding and promoter regions of TREH were sequenced in 39 Pima Indians, and 26 SNPs were genotyped in the 570 subjects who had measures of trehalase activity. Enzyme activity was highly associated with rs2276064 (Arg486Trp), rs10892251 (intron1), rs745663 (intron 13), and a novel SNP (intron 11) ($p=10^{-12}$ to 10^{-16}, adjusted for age, sex and family membership). Combinations of these SNPs explained 73-78% of the linkage signal ($p<0.0001$). To assess the potential role of these SNPs in BMI and T2D, all 26 SNPs were genotyped in the 1037 subjects that gave rise to prior BMI and T2D linkage peaks. Multiple SNPs were associated with T2D in these 1037 subjects ($p<0.0001$ adjusted for age, sex and family membership) and the association with T2D was replicated in a population-based sample of 3501 full-heritage Pima Indians (adjusted $p<0.0002$). Although we were unable to demonstrate that plasma/serum trehalase activity alone predicted onset of T2D, when haplotypes of rs2276064 (Arg486Trp) and rs10892251 were ordered according to their association with trehalase activity, there was a nominal association such that those haplotypes associated with the lowest trehalase activity were associated with the lowest prevalence of T2D ($P_{trend}=0.03$, adjusted for age, sex and family membership). These results indicate that SNPs within TREH strongly influence trehalase activity, and specific haplotypes of TREH are associated with both low levels of activity and risk of T2D.

Genetic Variants of *EGF* and *PPAR* are associated with neuroendocrine tumor risk and overall survival. *C. Liu*[1], *MH. Kulke*[2], *DC. Christiani*[1] 1) Dept Environmental Health, HSPH, Boston, MA; 2) Dana-Farber Cancer Institute, Boston, MA.

The molecular mechanisms of neuroendocrine tumor growth remain largely unknown. Lack of prognostic and predictive markers with high sensitivity has hindered the development of molecular targeted therapeutics and screening procedures. Common alleles of genes in several biological pathways, such as cell growth, cell signaling, hormones, and metastasis, may influence the outcomes of treatment and the risk of developing cancer. To identify susceptible genes for therapeutic effects of neuroendocrine tumor treatments, we conducted a large-scale evaluation of single nucleotide polymorphisms (SNPs) in association with overall survival and risk of neuroendocrine tumor among 274 cases and 329 age- and gender-matched controls. A total of 150 SNPs from 58 candidate genes were genotyped. The candidate genes were selected in the pathways of cell growth, cell signaling, hormones, and metastasis. The genotyped SNPs include missense, exonic, and SNPs in the promoter and UTR regions with minor allele frequency 0.05. To test the associations between genetic variants and overall survival, we used Cox proportional hazard model adjusting for age, gender, and grade of malignancy. 17 SNPs were found to be associated with overall survival of neuroendocrine tumor at p-value<0.05. Among them, a SNP on 5 UTR of *EGF* (epidermal growth factor) gene achieves global significance level. Adjusted hazard ratios (HR) and 95% confidence intervals (CI), are 2.3 (1.5-3.7), 2.2 (1.1-4.4), and 3.5 (1.8-6.9) for additive, dominant, and recessive genetic effect models, respectively. Using these 17 SNPs, we performed multiple logistic regression models adjusting for age and gender to test association with risk of neuroendocrine tumor. A SNP on the promoter region of *PPAR* (Peroxisome Proliferator Activated Receptor Gamma) was found to be associated with an increased risk (odds ratio:1.5; 95%CI:1.1-1.9)and the adjusted HR of overall survival is 1.7 (95%CI: 1.1-2.6). Replication of the SNPs on *EGF* and *PPAR* is on-going in an independent Caucasian population. In conclusion, we found the genetic variants of *EGF* and *PPAR* may be associated with neuroendocrine tumor risk and overall survival.

Pathogenesis of Loeys-Dietz Syndrome and Therapeutic Implications. *D. Loch, J. Chen, H. C. Dietz* Instit of Genet Med & HHMI, Johns Hopkins, Baltimore, MD.

Loeys-Dietz syndrome (LDS), caused by mutations in the type 1 or 2 subunits of the transforming growth factor-beta (TGF) receptor (Alk5 and TRII), shows aggressive arterial disease and frequent death in childhood, highlighting the need for the development of medical therapies through elucidation of disease pathogenesis. We find that aortic vascular smooth muscle cells (AoSMCs) from LDS patients show no defect in TGF signaling and that vascular specimens obtained at surgery consistently show nuclear accumulation of Smad2 that has been phosphorylated at the C-terminus (pSmad2; a mediator of the TGF signal) indicative of paradoxically enhanced TGF signaling. This is in keeping with the demonstrated role for TGF in aneurysm formation in Marfan syndrome and other disorders. TGF signaling is normally self-regulated by mechanisms that include induction of Smad7 and phophorylation of Smad2 at negative regulatory sites in the linker region that promote the clearance of activated receptors and signaling Smads, respectively. Despite clear evidence for increased TGF signaling *in vivo*, AoSMCs from LDS patients show low expression of Smad7 ($p<0.005$) and reduced linker phosphorylation of Smad2, suggesting that mutant receptors impair TGFs ability to induce autoregulatory programs. We also demonstrate a significant reduction ($p<0.005$) in expression of the TGF accessory receptor endoglin in LDS cells. TGF signaling normally induces endoglin, which in turn promotes utilization of Alk1 instead of Alk5 by TGF ligands, resulting in activation of the bone morphogenetic protein (BMP) cascade (pSmad1/5/8) and inhibition of the canonical TGF cascade (pSmad2/3). In keeping with failed induction of endoglin, LDS cells show reduced levels of pSmad1/5/8 ($p<0.01$). LDS AoSMCs show abnormally high levels of expression of selected vascular smooth muscle markers (smooth muscle actin and calponin), but fail to express terminal markers of VSMC differentiation such as smoothelin, suggesting that cells populating the aortic media may be myofibroblasts derived from pathologic endothelial-to-mesenchymal transition (EMT), a process normally inhibited by BMP signaling. We are currently testing TGF and EMT antagonists in recently created mouse models of LDS.

Genome-Wide Association Study Identifies Locus with Sex-Specific Genetic Effects on Serum YKL-40 levels in a Founder Population. *D. Loisel[1], Y. Sun[1], L. Pan[1], J. Elias[2], G. Chupp[2], C. Ober[1]* 1) Dept. of Human Genetics, University of Chicago, Chicago, IL; 2) School of Medicine, Yale University, New Haven, CT.

Asthma is a complex disease that shows striking sex differences in prevalence, age of onset, and severity. Serum levels of the chitinase-like protein YKL-40 are a biomarker for asthma, with elevated levels occurring in individuals with asthma and reduced lung function (Chupp et al. *NEJM* 2007; 357:2016). We conducted a genome-wide association study (GWAS) of serum YKL-40 levels in the Hutterites, a founder population of European descent, and identified the *CHI3L1* gene as a major locus for YKL-40 levels that also confers risk for asthma and reduced lung function in Caucasian populations (Ober et al. *NEJM* 2008; 358:1682). In the present study, we assessed the sex-specific genetic architecture of this highly-heritable biomarker for asthma and reduced lung function by testing for genotype-sex interactions on serum YKL-40 levels in the Hutterites, using the same GWAS data. 15 SNPs showed genotype-sex interactions at $P < 10^{-5}$. One SNP was located ~5kb downstream of a sex hormone receptor, making it an intriguing candidate locus for sex-specific effects in asthma and asthma-related phenotypes (genotype-sex interaction $P = 7.86 \times 10^{-6}$). We thus focused our study on this SNP and examined associations with an asthma-associated phenotype, bronchial hyperresponsiveness (BHR), in the Hutterites. The SNP showed a significant sex interaction with BHR ($P = 0.003$): males homozygous for the minor allele had the highest levels of serum YKL-40 levels and highest prevalence of BHR, while females homozygous for the minor allele had the lowest serum YKL-40 levels and lowest prevalence of BHR. The genotype association with BHR in males was significant ($P = 0.002$), although the association did not reach significance in females ($P > 0.10$). The results of this study highlight the value of examining genotype-by-sex interactions in the analysis of complex traits and diseases to identify novel genes. Supported by NIH grant HL085197.

LIMP-2 expression in fibroblast cell lines from subjects with Gaucher disease. *T. Samaddar, J. Vithayathil, N. Tayebi, E. Goldin, E. Sidransky, O. Goker-Alpan* MGB/NHGRI, NIH, Bethesda, MD.

Lysosomal integral membrane protein 2 (LIMP-2) is a specific chaperone responsible for trafficking glucocerebrosidase, the enzyme implicated in Gaucher disease, to the lysosome. In this study, LIMP-2 gene and protein expression were explored in 9 fibroblast cell lines from subjects with Gaucher disease using quantitative (real-time) PCR and Western blots. The mutations included N370S, L444P, D409H+, G325R, P415R and a recombinant allele The gene and protein expression varied between cell lines, even in the ones from subjects with the same genotype. In immunofluorescence studies, GC co-localized with the early endosomal marker EEA1, instead of the lysosomal markers. In these cell lines with trafficking defects, LIMP-2 distribution was altered and steady state protein levels were relatively decreased with respect to gene expression, suggesting over-utilization of LIMP-2 protein in an attempt to rescue mistrafficked GC. Incidentally, large endosomes were observed in the cell lines where GC was mistargeted to the endosome instead of lysosomes. In addition to its function as a membrane receptor for GC, LIMP-2 is also believed to regulate the post-endosomal compartment, and LIMP-2 overexpression in these fibroblast cell lines can lead to the formation of large endosomes. These results indicate that intracellular LIMP-2 regulation may differ depending on GC mutations and folding, and LIMP-2 could be a modifier molecule affecting the cellular and hence clinical phenotype in subjects with Gaucher disease.

Investigation of the P300 Evoked Response via Quantitative Trait Linkage Analysis in a Nepalese Population Genetic Isolate. *S. L. Santangelo[1,2], T. Sitnikova[1], J. Subedi[3], T. D. Dyer[4], S. Ojha[5], M. K. Nepal[5], P. Shrestha[6], S. Tamang[6], J. Blangero[4], J. VandeBerg[4], S. Williams-Blangero[4]* 1) Harvard Medical School, Psychiatric and Neurodevelopmental Genetics Unit, Center for Human Genetic Reasearch, Massachusetts General Hospital, Boston, MA, USA; 2) Harvard School of Public Health, Boston, MA, USA; 3) Miami University, Oxford, OH, USA; 4) Southwest Foundation for Biomedical Research, San Antonio, TX, USA; 5) Tribhuvan University Institute of Medicine, Kathmandu, Nepal; 6) Nepal Biomedical Research Center, Kathmandu, Nepal.

In an effort to map genes associated with schizophrenia, we have been measuring traits thought to be endophenotypes for schizophrenia in a population genetic isolate in Jiri, Nepal. Measured traits include P300, N100, N200 and P50 electrophysiological evoked potentials (ERPs), as well as a number of cognitive tests. The P300 ERP was recorded during an auditory "oddball" paradigm while participants identified infrequent target tones among standard tones by pressing buttons. P300 was measured at the Pz scalp location as the most positive peak between 280-600 msec after tone onset in 617 individuals in one large extended pedigree of over 2500 individuals in the Jirel population. P300 amplitude and latency were evaluated in a quantitative trait locus (QTL) genome-wide linkage analysis after estimating the heritability of each QTL. Although both were significant, the overall heritability of P300 latency (0.42; $p<0.0001$) was higher than that for amplitude (0.22; $p=0.0122$). QTL linkage analysis for P300 amplitude (transformed and adjusted for age, sex, age*sex, age^2*sex) yielded a peak LOD score of 1.58 on chromosome 21, and a LOD of 1.07 on chromosome 7. For P300 latency, there were peak LOD scores of 1.76 on chromosome 3, 1.43 on chromosome 13, 1.32 on chromosome 6, 1.28 on chromosome 20, and 1.17 on chromosome 11. In a genome-wide linkage scan, we did not find significant linkage for the P300 ERP as measured at Pz. As data collection is ongoing, we will continue to evaluate this and other QTLs in a larger sample from this Nepalese population isolate.

Increased non-right-handedness (NRH) and less hand lateralization in nonsyndromic cleft lip with or without cleft palate (CL/P). *K. Neiswanger[1], X. J. Wang[1], R. S. DeSensi[1], R. Martin[2], A. E. Czeizel[3], J. E. de Salamanca[4], M. L. Marazita[1]* 1) Univ Pittsburgh, Pittsburgh, PA; 2) Wash Univ, St. Louis, MO; 3) F Comm Ctl Hered Dis, Budapest, Hungary; 4) Hos Inf Univ Niño Jesús, Madrid, Spain.

Many individuals with nonsyndromic CL/P are NRH, which may reflect unusual brain lateralization. The Pittsburgh Oral Facial Cleft study assesses NRH as a phenotype that may increase the informativeness of CL/P families for gene mapping and identification. We analyzed age-appropriate items from a modified Edinburgh Handedness Inventory on 213 individuals with CL/P (115 male, 98 female), 452 relatives (196 male, 256 female), and 241 controls with no family history of clefts (87 male, 154 female) from Pittsburgh, St. Louis, Madrid, Hungary. We defined NRH at a laterality quotient score cutoff of 85, and degree of hand lateralization as follows: strongly lateralized subjects used only their right or left hand for every item on the inventory, while less lateralized subjects used both hands for at least one item. NRH was significantly increased in CL/P cases compared to unaffected relatives (58% vs 44% p=.001) and controls (58% vs 39% p=.0001). Almost half (45%) of CL/P subjects were less lateralized, compared to 34% of relatives (p=.006) and 25% of controls (p=.00001). Lateralization differences persisted in right-handers (RH)those who did not use their left hand alone to perform any task in the inventory. For RH, 43% of CL/P individuals were less lateralized, compared to 29% of relatives (p=.005) and 21% of controls (p=.00003). Relatives and controls differed significantly in their degree of lateralization (entire sample p=.02; RH p=.04). Overall, males showed more NRH (51% vs 42% p=.008) and less hand lateralization (p=.06) than females. Of the 129 subjects with unilateral CL/P, 89 had left-sided CL/P and 40 right-sided. The left-sided group had more NRH and less lateralization than the right (62% vs 50%; 45% vs 40%, respectively), but the differences were not significant. Our results confirm that NRH is elevated in CL/P individuals and further suggest that degree of lateralization, irrespective of hand preference, is lessened in CL/P families. NIH grants: R01-DE016148, R21-DE016930, P50-DE016215.

Allocation of YSTR Microvariant Alleles to Y-Chromosome Binary Haplogroups. *A. L. Pollock[1], K. Ritchie[1], P. A. Underhill[2], A. A. Lin[2], S. R. Woodward[1], U. A. Perego[1,3], N. M. Myres[1]* 1) Sorenson Molec Genealogy F, Salt Lake City, UT; 2) Department of Genetics, Stanford University School of Medicine, Stanford, California, USA; 3) Dip. di Genetica e Microbiologia, Università di Pavia, Italy.

Y-chromosome short tandem repeat (YSTR) loci are used extensively in studies of population substructure, temporality of population dynamics, and forensic identification. The occurrence of non-consensus YSTR alleles, such as unusually short alleles or partial insertion/deletion events (microvariants), have been used successfully as indicators of common ancestry among YSTR haplotypes, exposing further levels of phylogenetic substructure with restricted geographic distributions. However, the high variability of STR loci can potentially lead to false associations due to homoplasy (ie, recurrent mutation). Thus, YSTR haplotypes are best interpreted within the context of the binary marker defined Y-chromosome phylogeny. To identify YSTR microvariant alleles potentially useful for elucidating further phylogenetic substructure within binary haplogroups, we have assessed the haplogroup affiliation of microvariant alleles found at informative frequencies in public YSTR databases for the following YSTR loci: DYS385, DYS392, DYS441, DYS446, DYS447, DYS449 and DYS464. We report haplogroup affiliations for each variant allele and geographic origins of representative samples.

A genome-wide association study of gene expression in skin. *J. Ding[1], J. E. Gudjonsson[2], L. Liang[1], R. P. Nair[2], P. E. Stuart[2], J. J. Voorhees[2], J. T. Elder[2], G. R. Abecasis[1]* 1) Department of Biostatistics and Center for Statistical Genetics, University of Michigan, Ann Arbor, MI; 2) Department of Dermatology, University of Michigan, Ann Arbor, MI.

Gene transcript levels can serve as an intermediate phenotype that bridges genotypes and more complex organismal phenotypes. We carried out whole genome genotyping and gene expression analysis in a set of skin biopsies from 111 individuals (including a biopsy of normal skin from all 111 individuals, and a biopsy of affected skin from 54 individuals who had psoriasis). Altogether, we genotyped 450,652 single nucleotide polymorphisms (SNPs) and measured gene expression for 54,675 transcripts in each sample. Using whole genome association analysis, we identified 1,270 SNPs that are strongly associated with expression levels for one or more transcripts at $p < 10^{-8}$. Overall, 519 transcripts showed association at $p < 10^{-8}$ with at least one SNP and 1,244 showed association at $p < 10^{-5}$ with a SNP that mapped within 100 kb of the transcript. The results provide a genome-wide look at regulation of gene expression in skin and should aid interpretation of genetic association studies for many skin diseases. They also allow us to compare genetic regulation of gene-expression in skin to that in other tissues. Among the 1,562 SNP-transcript pairs that showed strong association at $p < 10^{-8}$ around 80% also showed association in an independent survey of gene expression in lymphoblastoid cell lines (Dixon *et al.*, *Nat.Genet.* **39**, 1202 (2007).). While the overlap in signal between tissues is much greater than expected by chance, our results emphasize the importance of studying how SNPs impact gene expression patterns in different tissues and cell lines.

The Autism Genome Project: Dissecting the genetic and genomic etiology of autism. *J. S. Sutcliffe for the Autism Genome Project* Vanderbilt Univ.

Autism is a neurodevelopmental disorder that affects approximately 1 in 150 individuals and is characterized by deficits in reciprocal social interaction, communication and patterns of repetitive behaviors and restricted interests. Twin and family studies indicate high heritability, but evidence supports a highly complex architecture for the underlying genetic etiology. The Autism Genome Project (AGP) was formed to facilitate gene identification by uniting investigators and family data. AGP Phase I involved genome-wide (GW) analysis of linkage and copy number variation (CNV) in >1100 multiplex families. Linkage analysis revealed promising loci on 11p and 15q, with gender and ancestry influencing signals at these and other loci. CNV analysis of 10k data showed a striking degree of CNV, with instances of both inherited and de novo CNV. AGP Phase II involves GW analysis for association and copy number using the Illumina 1M SNP array in a dataset to ultimately reach ~3000 families. We will present results from our analysis of >1000 AGP parent-child trios, alone and combined with data from >800 AGRE families genotyped for the 550k subset of markers (>1800 families total). *The AGP represents by far the largest genetic study undertaken for autism*. AGP genotyping sites sent 1M SNP data to the central Data Coordinating Center; raw data was distributed for CNV analysis, while genotype data was assessed for quality control (QC) and Hardy-Weinberg equilibrium (HWE) prior to analysis for association. In a preliminary CNV analysis of 370 families (1362 samples), multiple algorithms were utilized to infer CNV from intensity and genotype data, and 76,916 CNVs were called by at least two algorithms. Mean and median CNV sizes were 225kb and 32kb, respectively, and 57 CNVs were detected per proband on average. Family-based association analysis of 600 AGP families using 1M data alone, or combined with 800 AGRE families, does not reach GW-significance. Many genes identified by SNPs with $10^{-4} > P > 10^{-7}$ are related to neuronal development and guidance, similar to those identified by CNV. It seems possible then that risk for autism is due, in large part, to rare variation; this conclusion, however, awaits validation based on results from more samples.

Power for sequencing studies. *D. Hu, E. Ziv* Institute for Human Genetics, Comprehensive Cancer Center, Dept Medicine, Univ California San Francisco, San Francisco, CA.

Rare variants are increasingly being recognized as important determinants of complex traits. As the technology for identifying rare variants continues to improve, these studies will increasingly be used to discover the genes involved in complex traits. However, it remains unclear how such studies should optimally be designed. We analyzed the power for sequencing based case-control association studies. We used population attributable risk (PAR) of the cumulative effect of all variants identified. PAR is defined as the portion of the prevalence of a phenotype in the population that is due to all of the variants at the causative locus. We used population attributable risk because the relative risks associated with each variant are likely to vary from gene to gene and are too rare to be assessed independently. We defined power, as the probability of detecting a significant difference between the cumulative frequency of variants in cases and in controls. We used an alpha of 2.5×10^{-6}, adjusting for 20,000 genes tested. Our analyses demonstrate that, in general, for a particular PAR, genes with fewer variants are easier to be detected. We found that if the cumulative prevalence of functional variants in the population is 4%, we would have 80% power to detect a gene that has a PAR of 6.6% with a study that sequences 1000 cases and 1000 controls. In comparison, if the cumulative prevalence of functional variants in the population is 1%, we would have 80% power to detect a gene that has a PAR of 4.2% with the same sample size. Our results suggest that, in general, these studies will need large sample sizes, similar to the ones required for genome wide association studies.

Single Stem Cell Gene Expression Analysis on the SOLiD System. *K. Lao[1], F. Tang[2], C. Barbacioru[1], N. Xu[1], J. Gu[1], E. Nordman[1], M. Barker[1], R. Wicki[1], F. De La Vega[1], N. Straus[1], M. Surani[2]* 1) Molecular Cell Biology, Applied Biosystems, Foster City, CA; 2) Wellcome Trust/Cancer Research UK Gurdon Institute of Cancer and Developmental Biology, University of Cambridge, Tennis Court Road, Cambridge, CB2 1QN, UK.

Embryonic Stem Cells (ES cells) are a well-studied type of stem cells with pluripotency. Recently, it has been proved that undifferentiated ES cells are heterogeneous and are a mixture of stem cells at different status with differential marker gene expression. This raises importance of gene expression profiling analysis of small amount of pure population of stem cells, ideally of a single stem cell. To obtain gene expression information of a single stem cell on whole genome scale, we develop a deep sequencing assay of a single cell based on Applied Biosystems SOLiD System. This will permit us to get digital gene expression profiles of stem cells at single cell resolution and will enable us to understand the transcriptome landscape of all subpopulations of ES cells. The combination of next-generation sequencing using the SOLiD System and single cell cDNA technique will allow us to find all expressed transcripts in a stem cell, no matter whether it is from predicted/known genes or from unpredicted/novel genes. This will be of importance for stem cells because there are high chances that stem cells express some unique, novel transcripts. Because the SOLiD System provides a digital gene expression measurement, there should be no background noise and should cover a wider dynamic range of gene expression, which will make the expression profile more accurate. This is particularly interesting for stem cells because some of the key transcription factors are expressed at very low levels (below 20 copies per cell) in stem cells, which are very likely undetected using microarrays because of their significant level of noise.

Expression profiling study in human B cells identified novel miR-181b, miR-7 and miR-627 MicroRNAs underlying osteoporosis. *X. D. Chen[1], L. G. Sun[1], R. R. Recker[1], H. W. Deng[2], P. Xiao[1]* 1) Osteoporosis Research Center, Creighton University, Omaha, NE; 2) Department of Orthopedic Surgery and Basic Medical Sciences, University of Missouri-Kansas City, Kansas City, MO.

MicroRNAs (miRNAs) are short noncoding RNA molecules that regulate gene expression by targeting mRNAs and causing mRNA cleavage or translation blockage. Recently, miRNAs have been implicated important in the etiology of various diseases. Osteoporosis is characterized by low bone mineral density (BMD) mainly resulting from imbalanced osteoclastogenesis and osteoblastogenesis. B cells play important roles in osteoclastogenesis via expression of osteoclast-related factors, such as transforming growth factor beta (TGF) and osteoprotegerin (OPG). Our recent mRNA expression profile study on human B cells identified ER and MAPK3 gene network for postmenopausal osteoporosis. However, little is known about the role of miRNAs in human B cells for etiology of osteoporosis. In this study, we aimed to identify differentially expressed miRNAs in B cells between the low and high BMD groups. We recruited 20 unrelated postmenopausal Caucasian women aged 57-68, 10 with high BMD (spine or hip Z-score 0.84) and 10 with low BMD (spine or hip Z-score -0.84). For each subject, $CD19^+$ B cells were isolated from peripheral blood and total RNA (including miRNA) was extracted from the B cells. miRNA profiling for each sample was performed using TaqMan Low Density Array with 365 miRNAs. Expression level of each miRNA was determined with C_T by subtracting C_T of endogenous control RNU48. Differential miRNAs were selected by t-test for miRNAs expressed in all 20 samples and by Wilcoxon Rank Sum Test for miRNAs expressed in partial (20) samples. Three significant miRNAs, miR-181b, miR-7 and miR-627, were upregulated (p = 0.0479, 0.0337 and 0.0412, respectively) in the low vs. high BMD groups. It was reported that miR-7 inhibits epidermal growth factor receptor (EGFR), an important factor for osteogenesis. This is the first *in vivo* miRNA expression profile analysis in human B cells for osteoporosis. Our results suggested that miR-181b, miR-7 and miR-627 may be involved in osteoclastogenesis and etiology of osteoporosis.

Assessing the biological relevance of sequence variants in and around *DPYSL2*, a candidate schizophrenia (SZ) susceptibility gene on 8p21. *Y. Liu[1], D. M. McGaughey[1], L. Zhang[1], P. Chen[1], D. Avramopoulos[1], V. Lasseter[2], D. Fallin[3], J. McGrath[2], P. Wolyniec[2], G. Nestadt[2], K. Liang[4], A. Pulver[2], A. S. McCallion[1], D. Valle[1]* 1) Institute of Genetic Medicine; 2) Dept of Psychiatry, Johns Hopkins School of Medicine; 3) Dept of Epidemiology; 4) Dept of Biostatistics, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD.

Linkage and association studies from our group and others implicate *DPYSL2*, at 26.5Mb on 8p, as a candidate gene for SZ. We identified significant association with SNPs in intron2 (rs388047) in European Caucasian (CEU) probands and just 3 (rs12155555) of *DPYSL2* in Ashkenazi Jewish (AJ) probands. Expressed in both the CNS and PNS, *DPYSL2* (also known as *CRMP-2*) encodes a 62kDa cytosolic protein that regulates axonal growth. To identify additional variants in and around *DPYSL2*, we sequenced all exons (14), the proximal promoter and 8 cNCSs in and around *DPYSL2* in 48 CEU and 48 AJ SZ probands plus 48 AJ and 96 CEU controls. We found 2 missense and 2 synonymous coding variants whose frequency was similar in cases vs. controls. We also identified 3 SNPs (rs367948, rs400181, rs445678) in the promoter region and 2 SNPs (rs379266, rs11781865) in intron1 (all in cNCS), that were associated with SZ in the CEU sample only (p-values ranging 0.007-0.041). We assessed their biological relevance in cells (293 and primary E14.5 mouse cortical neurons) using a luciferase reporter and in zebrafish (ZF) larvae using Tol2 transposon-mediated transgenesis. For the promoter SNPs, we found 1.7-fold increase in expression with the risk alleles reaching statistical significance in primary E14.5 cortical neurons ($P < 0.0001$) but not 293 cells. In ZF, the intron1 cNCS directed tissue specific expression in the CNS and PNS. Expression in mosaic larvae was similar in the risk allele and the non-risk allele constructs. We conclude that non-coding variants in and around *DPYSL2* are associated with SZ possibly by regulating expression. We also identified an intronic enhancer in the region of the associated SNPs, but have not yet shown causality. Additional studies in cultured cells and ZF are in progress to further test the functional consequences of these variants.

Whole-Genome Genetic Difference in DNA Variation between Caucasians and Asians. *J. Li[1], T. B. Jin[2,3], Y. Z. Liu[1,2], C. Papasian[2], H. W. Deng[2,3,4]* 1) Info Med Person Health, Univ Missouri - Kansas City, Kansas City, MO 64108; 2) Orthopedic Surg/Basic Med Sci, Univ Missouri - Kansas City, Kansas City, MO 64108; 3) School of Life Science and Technology, Xi'an Jiaotong Univ, Xi'an, Shaanxi, 710049, PR China; 4) College of Life Sciences, Hunan Normal Univ, Changsha, Hunan, 410081, PR China.

Understanding differences of DNA variation across populations can provide insights and future research direction for studies on human diseases and human evolution. A powerful tool for this purpose is the high-throughput genotyping that becomes available recently. While studies on the ethnic differences of DNA variation have been performed, relative small sample sizes and ascertainment biases can compromise the reliability of the results. In this study, we targeted to provide a robust description of the similarity and dissimilarity of the genome-wide DNA variation across two ethnic groups, Caucasians and Asians. Our data contained dense single nucleotide polymorphisms (SNPs) based on Affymetrix GeneChip 500K Mapping array and consisted of almost 1700 study subjects. We started our study with the characterization of SNP sharing and SNP frequency distributions in the two ethnic groups. We then compared the genome-wide patterns of linkage disequilibrium (LD). We also studied haplotype sharing and the relationship between DNA variation and conservation scores in these two ethnic groups. Consistent with previous studies, we found that common SNPs in one population were often observed in the other ethnic group. However, the percentage of shared SNPs were higher, an indication of the effect of larger sample sizes in studying DNA variation. We also observed similar patterns of LD structure in these two ethnic groups. Our results enrich our understanding of the ethnic difference of DNA variation and potentially furnish more tools for future studies in human complex diseases.

SIX3 Mutation Studies Broaden Understanding of the Holoprosencephaly Clinical Spectrum. *B. D. Solomon, F. Lacbawan, B. Feldman, S. Domené, E. Roessler, M. Muenke* Medical Genetics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD.

Holoprosencephaly (HPE) is the most common structural malformation of the human forebrain. SIX3, which encodes a transcription factor expressed in the developing forebrain and eyes, is one of the most the common HPE-causing genes. In this largest cohort of patients (n=800), mutation screening of ZIC2, SHH, SIX3, and TGIF was done by PCR amplification of all exons, dHPLC screening, and was then followed by bidirectional sequencing. Among these genes, mutations in SIX3 (4.7%) were the third most common. Here we report 56 HPE cases with 38 different mutations in SIX3 and correlate their clinical manifestations. An additional 72 cases ascertained elsewhere with SIX3 mutations, submicroscopic deletions, or chromosome 2p21 abnormalities, are also included. The F:M ratio in this combined set of patients is 1.5:1. There is intrafamilial clinical heterogeneity in the 23 families, with penetrance of 68%. There are 27 patients with alobar HPE, 22 with semilobar, 7 with lobar, and 5 with MIHF. The most common associated clinical findings, in decreasing frequency, are hypotelorism, microcephaly, cleft lip/palate, seizures, premaxillary agenesis, diabetes insipidus, single central incisor, and coloboma. The majority of the mutations confer functional loss of SIX3, as clearly demonstrated by our group using an in vivo zebrafish assay (Domené et al., 2008), but the degree of severity of brain anomalies does not appear to be solely dependent on the genotype. However, there is regional clustering of the mutations within the SIX domain (43%) and homeodomain (26%) affecting the repressor function of SIX3. The related x-ray diffraction data on aa133-aa263 of SIX3 may be used in our attempts of molecular modeling to further refine genotype-phenotype correlations. As the understanding of the range of factors that could be functionally important is still incomplete, we cannot exclude important interactions between SIX3 and other genetic or environmental factors.

Ontology finger prints to quantitatively characterize genes and diseases for genome wide association study. *L. Tsoi[1], M. Boehnke[2], W. Zheng[1]* 1) Department of Biostatistics, Bioinformatics & Epidemiology, Medical University of South Carolina, Charleston, SC 29425-8350; 2) 2Department of Biostatistics, University of Michigan, Ann Arbor, MI, 48109-2029.

Genome Wide Association Studies(GWAS) may identify multiple genes that are associated with a disease. Quantitatively assessing how these candidate genes relate to the disease of interest is important to narrow down the candidate genes to perform downstream experimental validation. However, there is no widely acceptable way to assess quantitatively how genes may relate to disease, given that most diseases are complex and involve variants in many genes individually or combined. We developed an approach to assess if a gene is relevant to a disease by using ontology fingerprint derived from PubMed Abstracts. The fingerprints are generated by GO terms that are enriched in the abstracts relevant to a gene or disease through hypergeometric test. Such fingerprints can be quantified by the level of significance from the test, and the importance of a gene to a disease for GWAS can be assessed by comparing ontology fingerprints of genes to disease through the summary score calculation. We used 10 KEGG pathways to validate by comparing the summary scores of the genes belong to each pathway with that of genes from irrelevant pathways.We obtained a .9 for the averaged AUC of the 10 ROC curves,indicating the approach can reveal the relevancy of gene to biological phenomenon.By ranking the genes generated from GWAS on loci that influence lipid concentrations and risk of coronary artery disease, we found that the summary scores of this gene list are significantly higher than that of the rest of human genes, with larger proportion of GWAS identified genes obtaining higher score. This indicates GWAS-identified genes indeed has a significant bias toward genes involved in lipid metabolism. Our approach indicated that highest ranking genes are LDLR, APOE, and APOC2 for LDL; LIPC and CETP for HDL; and LPL for Triglyceride.We also find LPL, APOC2, and APOE are genes with highest ranking in all the above three studies.Our results demonstrate that ontology fingerprints can be used to effectively prioritize genes for GWAS.

Complex Disease Epistasis in AD: an enhanced version of MDR for complex SNP interaction analysis. *D. A. Ross[1], D. Wolfson[1], A. Smolgovsky[1], J. Sninsky[1], YH. Li[1], A. Grupe[1], J. H. Moore[2]* 1) Computational Biol, Celera, Alameda, CA; 2) Dartmouth College, NH.

Recently reported genome-wide association studies are using hundreds of thousands of SNPs and 1000s of patients and controls; these experiments are providing new opportunities to explore the epistatic network of genes that effect phenotypes. We have revised the program MDR, multifactor-dimensional-reduction(we call it MyT-MDR)so that it can accommodate datasets with hundreds of thousands of SNPs and 1000s of patients. Normalized-mutual-information and other metrics were incorporated for model evaluation. A significant speed increase, 12X per unit node was achieved by code refactoring; the code runs on a Beowulf-Scyld parallel cluster. The improved speed allowed us to perform the initial analysis and repeated cross-validation (10 fold CV with 10 fold randomization) within 5 days using whole genome SNP data and thousands of individuals. Cross validation was key to achieving an accurate prediction of models which will replicate(Motsinger and Ritchie (2006). When using synthetic data-sets with heritability of .05 we can repeat cross-validated studies for the 300K SNPs and 2000 individuals to obtain 3 and 4 way interactions. We use a small set of randomizations of the phenotype with patients to obtain a distribution of the false discovery rate to help evaluate the models. To explore interaction analysis in real data-sets we used the AD dataset from TGEN (Reinman, E. et. al. (2007) Neuron 54:713); in which the initial univariate analysis identified a single marker system around GAB2. This dataset has approximately 1400 case/controls. The dataset was divided into a train-test discovery(75%) and validation(25%)data-sets. The discovery set was repeat-cross-validated 100 fold to identity SNPxSNP models that were evaluated for error in the testing partitions. Finally a small number of these models were evaluated in the further validation datasets. We present significant marker models which replicated through all datasets. Although the overall size of the initial dataset was small, larger sample sizes can be handled efficiently using MyT-MDR to identify SNPxSNP interactions.

Asperger syndrome: atypical phenotype trisomy 10q. *L. R. J. da Silva, N. Sobreira, D. Brunoni, C. Borovik, A. Perez*
Morfology, UNIFESP, São Paulo, São Paulo, Brazil.

Asperger syndrome (AS) is a form of pervasive developmental disorder characterized by impairment in social interaction as well as a restricted pattern of behavior, interests, and activities. It is diagnosed almost exclusively in individuals with normal or high level of intelligence. The estimated male-to-female ratio is approximately 4:1. The pathophisiology of Asperger disorder is unknown but a susceptibility region was suggested, by Tentler et al. (2002), on 17p13 based on molecular characterization of two breakpoints. We describe a 16 years old male, only son of non consanguineous parents. To the birth he presented P=3400g, E=51cm, of scapular waist and criptorquidia at left side. He presented delayed milestones and abnormal behavior. His physical examination presents long face, long eyelashes, high and narrow palate, narrow and long thorax, long fingers (P>P97), increased distance between halux and 2º toe and overlapping of 3º on 2º and 4º toes, absence of plantar creases, cifoscoliosis, muscular hypotrophy and tall stature (P>97). He still presents infantile and effeminate behavior and increased interest for determined subjects. The echocardiogram evidenced diameter of aorta in the superior limit. The psicodiagnosis confirmed Asperger syndrome. Karyotype showed the presence of an additional segment in the short arm of chromosome 17, 46, XY, add(17p), and karyotype of the parents was normal. Using array CGH (GenoSensor, Abbott, Inc.) the additional segment was identified as 10q26-10q26.3 region. Distal trisomy 10q is a well know, but rare, syndrome. Typical features consist of psychomotor delay, a distinctive dysmorphic appearance, and growth retardation. This patient represents a rare case of pure distal trisomy 10q and does not presents the typical characteristics of this syndrome. On the other hand he presents tall stature and Asperger syndrome and candidates genes to Asperger syndrome were suggested at 17p13. So the comparation between the duplicated regions and the evaluation of the expression of genes in these regions of chromosome 10 and 17 may reveal candidate genes for these phenotypes.

Renal transplant in methylmalonic acidemia: a therapeutic option with or without renal failure. *P. de Lonlay[1], D. Rabier[2], G. Guest[3], Y. Aigrain[4], JF. Benoist[5], P. Niaudet[3], V. Valayannopoulos[1]* 1) Metabolic Dept, INSERM U393,Université Paris Descartes Hosp Necker-Enfants Malades, Paris , France; 2) Biochem Lab, Hosp Necker-Enfants Malades, Paris , France; 3) Ped Nephrology, Hosp Necker-Enfants Malades, Paris , France; 4) Ped Surgery, Hosp Necker-Enfants Malades, Paris , France; 5) Biochem Lab, Hosp Robert Debré, Paris , France.

Background: In methylmalonic acidemia (MMA), liver, kidney, or combined liver and kidney transplantation is a possible therapeutic option. Case Report: We report on a 5-year-old boy with MMA. The first symptoms occurred at 4 days of life, namely hyperammoniemic coma and ketoacidosis. After medical treatment his clinical course was uneventful until the 2nd year of life when he displayed several episodes of metabolic decompensation with vomiting and failure to thrive that required continuous enteral feeding. His neurological condition deteriorated (tremor, developmental arrest) while MMA levels remained high. We decided to propose renal transplantation for this patient despite normal renal function because liver transplantation was considered at high risk because of his impaired metabolic condition. Results: The procedure was uneventful and MMA levels in blood and urine fell rapidly to very low levels. Ten months after the transplant the patient is at home, started oral feeding and has normal somatic and cognitive developments and moderate protein restriction. No further metabolic decompensation occurred, except from a single and short episode of acute encephalopathy with low urine and plasma MMA levels, neither hyperammoniemia nor ketoacidosis, but elevated MMA levels in CSF. Conclusion: Although liver is the major site of methylmalonyl-CoA mutase activity, this case and similar ones suggest that the smaller mutase activity present in the transplanted kidney may be sufficient to ensure partial correction of the metabolic defect. However organ transplantation does not prevent neurological complications where the blood brain barrier by trapping toxic metabolites may play a major role.

Genome-wide Linkage Analysis of Colorectal Cancer in 302 Multiple-Case Families. *E. L. Goode[1], B. L. Fridley[1], W. Bamlet[1], D. Serie[1], R. W. Haile[2], D. C. Thomas[2], S. N. Thibodeau[1], N. M. Lindor[1], J. D. Potter[3] for the Colon CFR*
1) Mayo Clinic, Rochester, MN; 2) University of Southern California, Los Angeles, CA; 3) Fred Hutchinson Cancer Research Center, Seattle, WA.

Even accounting for known syndromes, colorectal cancer risk is associated with family history, and patterns of inheritance suggest other major susceptibility loci. We assessed linkage in 302 families of the Colon CFR, a multi-site NCI-supported consortium; families known to have FAP, MAP, or Lynch-related mutations were excluded. 1,882 individuals were successfully genotyped using the Affymetrix 10k 2.0 Array (5,453 SNPs with $r^2<0.1$) including 703 cases. Among 287 Caucasian families, peak Kong & Cox LOD scores (KCL) of 2.11 on chr 3 (128.8 cM), 1.60 on chr 23 (329.7 cM), and 1.40 on chr10 (308.7 cM) were observed. In addition, dominant lods (DL) of 7.27 on chr 2 (109.2 cM; 41% of families linked), 2.20 on chr 13 (41.1 cM; 26%), 1.53 on chr 15 (39.9 cM; 26%) were seen, assuming heterogeneity. Analysis of 30 families with loss of MLH1 expression yielded a KCL = 2.20 in the chr 3 *MLH1* region (97.5 cM; DL = 3.69, 94.8 cM) as well as a KCL = 2.42 on chr 19 (86.9 cM). Analysis of 18 families with MSH2 loss, including 17 with MSH6 loss, yielded a KCL = 5.07 in the *MSH2-MSH6* region (chr 2, 114.6 cM; DL = 10.3, 111.87 cM). 180 families were screened for MSI; the peak DLs for families with MSI-H (N=59), MSI-L (N=32), and MSS (N=89) tumors were 0.77 (*MLH1* region, chr 3, 94.81), 1.61 (chr 21, 55.8 cM), and 1.02 (chr 1, 205.8 cM), respectively. Among 219 families that did not have loss of MMR expression or MSI-H tumors, the genome-wide peak KCLs were 1.22 (chr 12, 182.6 cM), 1.17 (chr 3, 128.1 cM) and 1.00 (chr 5, 289.8 cM) with DLs of 1.58 (chr 4, 148.8), 1.38 (chr 2, 175.0), and 1.35 (chr 1, 195.7 cM), assuming heterogeneity. These results confirm linkage to *MLH1*, *MSH2*, and *MSH6* for families with protein loss and suggest novel regions for those with MMR proficiency. Among the latter group, minimal power and residual heterogeneity emphasize the need for analysis of newly-recruited families, combined analyses with other studies, and application of approaches to integrate linkage and association scans.

Detecting Inversions in the Human Genome from SNP data. *P. F. O'Reilly, C. J. Hoggart, L. J. Coin* Epidemiology & Public Health, Imperial Col London, London, United Kingdom.

The importance of structural variation in the human genome, in terms of its ubiquity and association with disease, has only recently been established. Since the first genome-wide map of structural variation by Redon et al (2006), intense efforts have focused on identifying Copy Number Variants (CNVs). The discovery of inversion polymorphisms has been relatively minimal, largely because of the particular challenge that their detection presents. However, recent evidence that inversions may be common in the genome, and examples suggesting a role in disease causation, call for the development of powerful methodology to identify them from large-scale SNP data. Here we present a statistical method, based on a Hidden Markov Model (HMM), designed to scan the genome for inversions using genotype data. The HMM is constructed to highlight loci where there exists a bipartition of individuals showing no, or little, between-group recombination. This captures the main discernible feature of inversions from genotype data: that recombinations between the inverted and non-inverted types are suppressed. Using an extensive forward-in-time simulator we find that our method has good power to detect even small inversions, and outperforms current alternatives. We apply our method to several large human datasets, building the most comprehensive genome-wide map of candidate inversions so far.

Mutations in the glucocerebrosidase gene confer a five fold increased risk of developing Parkinson disease: Results of an international multi-center collaborative study. *E. Sidransky, Multi-Center Collaborative Group Studying GBA Mutations in PD* MGB, NHGRI, NIH, Bethesda, MD.

Recent studies have demonstrated an increased frequency of mutations in the gene encoding glucocerebrosidase (GBA), the enzyme deficient in Gaucher disease, among different cohorts with parkinsonism. To better establish the frequency of GBA mutations in ethnically diverse subjects with Parkinson disease (PD), and to ascertain the relative risk of developing PD in GBA mutation carriers, we assembled an international multicenter collaborative group of investigators screening for mutations in this gene. As a first step, each group was provided with a uniform panel of DNA samples to determine which mutations could be detected by their center. Next, genotypes and phenotypic data on patients and matched controls were collected and assembled. The study included four centers from North America, two from South America, six from Europe, two from Israel and three from Asia, and included a total of 5749 patients (780 Ashkenazi Jews) and 4840 controls (399 Ashkenazi Jews). All participating centers demonstrated the ability to detect GBA mutations L444P and N370S. These two mutations were found in a total of 287 patients (5.0%), including 119 Ashkenazi Jews (15.3%) and 168 non Ashkenazim (3.4%), and 42 controls (0.87%), including 15 Ashkenazi Jews (3.8%) and 27 non Ashkenazim (0.61%). Screening for a total of 7 mutations in the Ashkenazi Jewish group increased the mutation frequency to 19.7% in patients versus 4.5% in controls. Among the non-Ashkenazi Jewish subjects, sequencing of all GBA exons was performed in a total of 1794 patients and 1446 controls, yielding a mutation frequency of 7.1% and 1.6% respectively, indicating that screens for only the L444P and N370S mutations in non-Ashkenazi cohorts may miss as many as 50% of mutant GBA alleles. Phenotypic data is being compared between heterozygous GBA mutation carriers and patients with wildtype GBA alleles. Our data demonstrate that the relative risk of developing PD in GBA mutation carriers from diverse ethnicities is increased approximately five-fold, rendering it one of the most significant PD risk factors indentified to date.

INCREASING THE EFFICIENCY OF GENOMEWIDE ASSOCIATION MAPPING VIA HIDDEN MARKOV MODELS. *H. Gao[1], H. Tang[1], C. D. Bustamante[2]* 1) Department of Genetics, Stanford University School of Medicine, Stanford, CA; 2) Department of Biological Statistics and Computational Biology, Cornell University, Ithaca, NY.

With the rapid production of high dimensional genetic data, new difficulties have arisen in genome-wide association studies. A major one is the lack of effective and efficient statistical tools to resolve the loss-of-power problem caused by performing multiple statistical tests across millions of SNPs simultaneously. Here we present a novel method that serves as an effective measure for balancing power and type I error in genome-wide association studies. The approach uses hidden Markov model and its derivate Markov hidden Markov model to estimate the posterior probabilities of a markers being in an associated state, which naturally utilizes the correlation information among consecutive markers. We conducted extensive simulations based on the whole genome genotype data (500K SNPs) of 1000 Europeans from the GlaxoSmithKline project to calibrate the sensitivity and specificity of our method. We compared our method with many popular approaches for correcting multiple tests including Bonferroni correction, false discovery rate (FDR) control or positive false discovery rate (pFDR) control and Q-value control. Our simulation results suggested that at comparable low false positive rates 10^{-5}, our method is significantly more powerful than many other approaches, given moderate susceptibility of disease-predisposing variants.

Contractility of individual myofibers is altered in individuals with myosin mutations that cause Freeman-Sheldon syndrome. *A. E. Beck[1], M. J. McMillin[1], F. S. Korte[2], A. W. Ward[2], M. Regnier[2], M. J. Bamshad[1]* 1) Dept Pediatrics, University of Washington, Seattle, WA; 2) Dept Bioengineering, University of Washington, Seattle, WA.

The distal arthrogryposis (DA) syndromes are a group of ten dominantly inherited disorders characterized by multiple congenital contractures such as clubfoot and camptodactyly. DA syndromes are caused by mutations in at least seven different genes that encode components of the contractile apparatus of fast-twitch myofibers including troponin I, troponin T, tropomyosin, and several myosin heavy chains. *In vitro* contractility studies using recombinant mutant troponin and tropomyosin molecules suggest that DA syndromes are, in some cases, caused by increased myofiber contractility, although the mechanism by which contractility is altered is disputed. Mutations in *TNNI2* and *TPM2* explain only a small fraction of DA cases. The majority of mutations found to date to cause DA syndromes are located in *MYH3*, the gene that encodes embryonic myosin. Non-overlapping mutations in *MYH3* cause both the most common DA, Sheldon-Hall syndrome (SHS), and the most severe DA, Freeman-Sheldon syndrome (FSS). This observation suggests that *MYH3* mutations causing FSS have different functional consequences than those causing SHS. Myosin molecules are challenging to manipulate *in vitro* so we elected to explore the relationship between myosin mutations and muscle contractility by directly measuring the contractile properties of single chemically skinned muscle fibers sampled from affected muscles in individuals with FSS. Preliminary analysis suggests that maximal force normalized to fiber cross-sectional area was similar in case and control fibers, but the relationship between calcium concentration and contractile force differed between cases and controls. Understanding the mechanism by which myosin mutations affect myofiber contractility could provide a model to explore the mechanisms underlying more common contractures such as idiopathic clubfoot and facilitate the development of novel therapeutic approaches.

Clinical Genetics of Moebius Syndrome. *H. Gaspar[1], S. Chang[2], S. Scott[1], L. Edelmann[1], E. W. Jabs[1], . Moebius Syndrome Foundation[3]* 1) Mount Sinai School of Medicine, New York, NY; 2) Johns Hopkins University, Baltimore, MD; 3) Pilot Grove, MO.

Moebius syndrome is a rare, congenital disorder with partial or complete agenesis of the 6th and 7th cranial nerves (CNs), which control eye movements and facial expression. There is a broad spectrum of associated features such as limb malformation and mental retardation. This heterogeneous condition is usually sporadic, but can be inherited in a dominant or recessive manner. Hereditary Congenital Facial Paresis involves only the 7th CN and loci have been identified at 3q21-q22 and 10q21.3-q22.1. Our goal is to better define the diagnostic criteria and delineate the spectrum of features associated with Moebius syndrome by studying the largest reported cohort of 74 patients with a minimum criterion of 7th CN involvement. Clinical information was obtained from a questionnaire, medical records, and physical examinations on patients primarily referred by the Moebius Syndrome Foundation. Our data showed that 59 patients had the classic features of 6th and 7th CNs (80%) and 15 atypical patients had only 7th CN involvement (20%). We observed 3 dominant and 4 autosomal recessive families. Forty-seven patients (64%) had involvement of other CNs; 48 (65%) had muscular hypotonia; 37 (50%) had developmental delay in motor skills, 6 (8.1%) had mental retardation, 4 (5%) had seizures, 2 had autistic spectrum disorder, 3 (4.1%) had peripheral neuropathy, 47 (64%) had limb abnormalities, 7 (10%) had Poland sequence and 8 (11%) had cardiac defects. Moebius patients with both, 6th and 7th CN involvement showed a broader spectrum of features, particularly with neurological and cardiac findings, whereas familial cases were more common in patients with only 7th CN involvement. Karyotyping revealed an unbalanced de novo translocation chromosome t(10;15) in one patient and a familial balanced t(2;11) in another patient. By array CGH we did not find consistent aberrations in 20 complex cases, but we did identify specific aberrations in multiple patients involving regions of chromosomes 5, 7, 14 and 15. Other patients screened for candidate genes HOXB1, HOXB2, and KROX20 did not yield mutations.

Genotype/phenotype correlation in two Brazilian families harboring the Cys433Arg mutation in the MYOC gene. *J. P. C. Vasconcellos[1], R. Reis[2], C. Stefani[3], A. Tavares[4], C. A. Braghini[4], P. R. S. Cruz[4], D. B. O. Santos[4], S. R. E. Pião Jr.[4], V. P. Costa[1], A. V. Celestino[4], M. B. Melo[4]* 1) Dept. Ophthalmology, University of Campinas - UNICAMP, Campinas SP, Brazil; 2) Instituto da Visão, S. J. Rio Preto SP, Brazil; 3) Braojos Oftalmologia, S. J. Rio Preto SP, Brazil; 4) CBMEG, University of Campinas - UNICAMP, Campinas SP, Brazil.

Introduction: Primary open angle glaucoma (POAG) is characterized by progressive excavation of the optic disc with corresponding visual field loss. Mutations in the MYOC gene account for most cases of autosomal dominant juvenile open angle glaucoma (JOAG), an earlier and more severe form of POAG. In Brazilian JOAG patients, the most prevalent alteration associated with the disease is the Cys433Arg. Two JOAG Brazilian families harboring this mutation have been evaluated according to clinical parameters. Methods: A comprehensive ophthalmic examination was performed, including intraocular pressure (IOP) measurement, optic disc and corneal thickness evaluation. POAG was defined as untreated IOP over 21 mmHg, with characteristic optic nerve and visual field glaucomatous damage. Results: Twenty two out of 53 members of the families harbor the Cys433Arg mutation (9 developed glaucoma, 5 have high IOP and 8 are normal). Among the normal individuals with mutation, two members of the same family have more than 58 years of age. The mean IOP in right eyes (RE) was 16.203.60 and 27.5513.03 in the absence and presence of the mutation, respectively (P=0.002). In left eyes (LE) the mean IOP was 16.413.55 and 28.5814.37 in the absence and presence of the mutation, respectively (P=0.002). Cup to disk ratio in RE was 0.140.07 in eyes without mutation and 0.390.33 in eyes with mutation (P=0.003) and in LE it was 0.150.07 and 0.410.35 in the absence and presence of mutation, respectively (P=0.003). Corneal thickness did not differ in both groups. Conclusions: The Cys433Arg mutation in these two families is associated with high intraocular pressure, augmented cup to disk ratio and early onset glaucoma. The incomplete penetrance after 58 years of age in one of the families suggests the possible role of other genes modulating the disease phenotype.

Genome-wide Analysis of Gene-Gene Interaction in Alzheimer Disease. *S. D. Turner[1], E. R. Martin[2], G. W. Beecham[2], J. R. Gilbert[2], J. L. Haines[1], M. A. Pericak-Vance[2], M. D. Ritchie[1]* 1) Center for Human Genetics Research, Vanderbilt University, Nashville TN; 2) Miami Institute for Human Genomics, University of Miami, Miami, FL.

Genome-wide association studies (GWAS) have revolutionized complex disease studies by identifying individual genes that previously resisted identification. However, these genes rarely explain more than a few percent of the overall genetic risk. To more fully exploit the richness of these datasets and explore alternatives to single gene effects, we examined the feasibility of testing for gene-gene interactions using an Alzheimer Disease (AD) GWAS dataset of 492 AD cases and 496 cognitively normal controls. Single gene studies of this and other AD GWAS datasets confirmed that the only strong effect in AD is APOE. To identify novel interactions we removed APOE from the dataset and performed multifactor dimensionality reduction (MDR) analysis on the first half of the dataset. 13 two-locus models with a cross-validation consistency (CVC) of 9 or 10 were identified. Regression analysis of these 13 models in the second half of the dataset confirmed two strong interactions, one between rs12683393 (PRG-3 on chromosome 9) and rs3791426 (HDAC4 on chromosome 2) (OR=2.44, p=0.034) and another between rs6473522 (on chromosome 8) and rs11265191 (OR10J on chromosome 1) (OR=2.93, p=0.006). These interaction effect sizes are substantially larger than any single locus effect other than APOE. To test for interactions with APOE, we ran MDR on the full dataset (to increase power given APOEs strong main effect) and identified a model with high prediction accuracy (72.3%) that included APOE and rs2161082, an intronic SNP in SPAG16 on chromosome 2. These results suggest that gene-gene interaction effects in AD are substantially larger and perhaps more important than individual gene main effects. They also highlight the value of deeply exploring the richness of GWAS data.

Very-long-chain acyl-CoA dehydrogenase deficiency in two patients with normal newborn screening by tandem mass spectrometry. *C. Ficicioglu, C. R. Coughlin II, M. Yudkoff* Biochemical Genetics, Children's Hospital of Philadelphia, Philadelphia, PA.

Very-long-chain acyl-CoA dehydrogenase deficiency (VLCADD) is an autosomal recessive disorder of fatty acid oxidation (FAO). VLCADD may present with cardiomyopathy in the neonatal period, hypoglycemia in childhood, and rhabdomyolysis and myglobinuria in adulthood. VLCADD can be detected through newborn screening by measuring C14: 1 level with tandem mass spectrometry. Case 1:The first patient was a previously healthy nine-month old infant presented with severe hypoglycemia and dehydration after a one-day history of acute gastroenteritis. She developed hypoxic ischemic encephalopathy and liver failure, and died as a result of severe brain injury due to hypoglycemia. The extensive metabolic work up did not show any specific disease. Plasma acylcarnitine analysis showed elevations of multiple long-chain acylcarnitine species in a non-disease specific pattern. Newborn screening was within normal limits with a C14: 1 of 0.58 umol/L .The patients sibling was born three years later and newborn screening revealed an elevated C14: 1 level of 2.21 umol/L. Plasma acylcarnitine analysis performed on day two of life revealed C14 and C16 elevations that were consistent with diagnosis of VLCADD. Sequence analysis of the VLCAD gene revealed two novel mutations (1049G>T, 1700G>A). These mutations were later confirmed in the deceased patients fibroblast. Case 2: The second patient presented at 13 months of age as a result of hypoglycemia leading to an unresponsive state. Prior to this episode she was hospitalized twice for dehydration secondary to vomiting and diarrhea. Acylcarnitine analysis performed during her presenting illness revealed C14 and C16 elevations that were consistent with VLCADD. Sequence analysis of the VLCAD gene revealed two mutations (IVS7-8C>T, 1600G>A). Her newborn screen was within normal limits. Our cases show that newborn screening may not detect all cases of VLCADD. A diagnosis should be considered when clinical symptoms are consistent with a disorder of FAO even if newborn screening and acylcarnitine analysis are within normal limits.

I Gave at the Office: Privacy, Discrimination, and the Employer-Sponsored Biobank. *L. R. Eisenberg, J. M. Henriksen Hellyer, B. A. Koenig* Bioethics Research, Mayo Clinic, Rochester, MN.

Giving at the office used to refer to a donation to the United Way or the purchase of Girl Scout cookies. Giving at work may soon take on new dimensions as more research institutions develop biobanks stocked with DNA samples from healthy members of the population. It is inevitable that staff persons at research institutions will be asked to donate blood to employer-sponsored population-based biobanks. We explore the concerns that arise when an employer maintains a biobank that not only stores biological samples but also associates them with a wide range of health, lifestyle, and ancestral information. A critical issue in the maintenance of any biobank is privacy. Although samples are separated from identifying information such as name and social security number, its unclear whether complete anonymity is possible in the relatively small community of a research institution. Despite the recent passage of the Genetic Information Nondiscrimination Act, there is a widespread fear that employers will try to control costs in the future by limiting health coverage based upon an individuals genetic makeup. A recent study found that most adults would trust researchers to properly handle their genetic information but would not trust their employers with the same data. Which belief will prevail when employer and researcher are one in the same? How can an employer earn and keep the employees confidence while avoiding coercion stemming from the power differential inherent in their respective roles? Institutions committed to maintaining trust must apply intentional strategies when designing and implementing population-based biobanks. We examine such strategies, including the use of a deliberative democracy process, which involves employees in active, reasoned reflection about the risks and possibilities of biobanking in the design stages of the project, as well as continued input via consultation roles for the employee-participants as part of ongoing advisory groups. Biobanks may also utilize after-the-fact methods for improving privacy, such as the use of a charitable trust, or the double coding of samples with the help of a trusted intermediary.

Genome-Wide Association of Bipolar Disorder in European American and African American Individuals. *C. S. Bloss[1], E. N. Smith[1], C. Nievergelt[3], N. J. Schork[1,2], J. R. Kelsoe[3] for The Bipolar Genome Study (BiGS) Consortium* 1) Scripps Genomic Medicine, Scripps Translational Science Institute, La Jolla, CA; 2) Department of Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla, CA; 3) Department of Psychiatry, University of California, San Diego, La Jolla, CA.

Bipolar disorder (BD) is a debilitating neuropsychiatric illness characterized by alternating episodes of depression and mania. Family, twin, linkage, and candidate gene studies all suggest a strong genetic component in BD, although genome-wide association (GWA) studies, which to date have been limited to individuals of European Ancestry (EA), have generally failed to detect signals that have been replicated consistently. We performed two GWA studies, one in a sample of individuals of EA (n = 1,001 cases; 1,034 controls) and the second in a sample of individuals of African Ancestry (AA; n = 362 cases; n = 671 controls). Both studies utilized the Affymetrix 6.0 array. Analyses involved association using different methods to adjust for admixture, as well as exploration of the extent to which some single nucleotide polymorphisms (SNPs) demonstrate genetic background-dependent effects. Results revealed significant levels of admixture, particularly among individuals of AA, and highlight the need for analytic methods that account for this. Among individuals of EA, six SNPs in the Nck-associated protein 5 (NAP5) gene were nominally associated with BD (p-values all $< 1 \times 10^{-5}$), a gene known to be expressed in both fetal and adult human brain tissue. Among individuals of AA, three SNPs in the dyp-19-like protein 3 (DPY19L3) gene were nominally associated with BD (p-values all $< 1 \times 10^{-5}$), a gene with evidence of expression in several human tissues including brain. We also considered analyses exploring copy number variations and pathways implicated by the most strongly associated variations. Overall, our study is among the first to conduct GWA of BD in individuals of AA and suggests that genetic variations that contribute to BD may vary as a function of ancestry.

Sequence variation in the *IL4* gene and resistance to *Trypanosoma cruzi* infection in Bolivians. *L. E. A. Arnez[1], E. N. Venegas[1], C. Ober[2], E. E. Thompson[2]* 1) Laboratory DIANA of Histocompatibility and Immunogenetics, Cochabamba, Bolivia; 2) Department of Human Genetics, The University of Chicago, Chicago IL.

Th2 immunity plays a central role in response to intracellular pathogens, such as *Trypanosoma cruzi* (*T. cruzi*), which is endemic to Latin America. Variation in the gene encoding interleukin 4 (IL-4), a signature Th2 cytokine, has been associated with Th2-mediated diseases. A promoter polymorphism (-590C/T; rs2243250) affects gene expression and has been associated with susceptibility to Th2-mediated allergic and parasitic diseases. We hypothesized that variation in the *IL4* gene confers differential resistance to T. cruzi infection. The *IL4* gene was re-sequenced in DNA from 110 Bolivians to determine whether genetic variation at this locus contributes to *T. cruzi* infection status in exposed individuals. Standard sequencing techniques were used to detect single nucleotide polymorphisms (SNPs) in susceptible (*T. cruzi* positive cases) and resistant (exposed but *T. cruzi* negative controls) individuals from Bolivia. A total of 45 polymorphisms were identified in the *IL4* gene, 19 of which have not previously been reported, including a novel non-synonymous SNP. Two classes of haplotypes, referred to here as Clade A and Clade B, defined by alleles at a functional promoter SNP, -590C/T, were identified. Using the chimpanzee as an outgroup, we estimated that these haplotypes diverged approximately 40,000 years ago. Clade A haplotypes (with the derived -590T allele) were more common in the resistant group compared to the *T. cruzi* susceptible group (0.64 v. 0.49, respectively, $P = 0.033$). We also observed an excess of intermediate frequency variants in the resistant group (Tajimas D =1.78, $P = 0.01$) and the occurrence of all singletons in the susceptible group ($P = 0.0025$), suggesting that selection is favoring the maintenance of Clade A haplotypes in the Bolivian population. Our study of the *IL4* gene in Bolivians reveals many alleles that may be population-specific, and the possible involvement of a common haplotype conferring protection against *T. cruzi* infection. This work was supported by NIH grants R01 HL072414 to C.O. and T32 HL007605 to E.E.T.

Fine mapping on chromosome 10q22-23 implicates *Neuregulin 3* (*NRG3*) in Schizophrenia. *P. Chen[1], D. Avramopoulos[1,2], V. K. Lasseter[2], J. McGrath[2], M. D. Fallin[3,4], K.-Y. Liang[4], G. Nestadt[2], N. Feng[1], G. Steel[1], A. S. Cutting[1], P. Wolyniec[2], A. Pulver[2], D. Valle[1]* 1) Inst Genetic Medicine, Johns Hopkins Sch Medicine, Baltimore, MD; 2) Dept Psychiatry, Sch of Medicine; 3) Dept Epidemiology, Sch of Public Health; 4) Dept Biostatistics, Sch of Public Health.

Linkage studies have implicated 10q22-23 as a schizophrenia (SZ) susceptibility locus in Ashkenazi Jewish (AJ) (Fallin *et al*. Am J Hum Genet 73:601, 2003) and Han Chinese from Taiwan (Faraone *et al*. Am J Psychiatry 163:1760, 2006). To further explore our previous linkage signal in the AJ (NPL score: 4.27, empirical $p = 2x10^{-5}$), we performed a peakwide association fine mapping study using 1414 SNPs across ~12.5 Mb in 10q22-23 in 1515 AJ individuals, including 285 parent-child trios, 173 unrelated cases and 487 unrelated controls. We analyzed the binary diagnosis of SZ and 9 heritable quantitative traits derived from a principal components factor analysis of 73 items from our consensus diagnostic ratings and assessment interviews. Although there was no association with the diagnosis of SZ, we found strong evidence of association using the Delusion factor as the quantitative trait at 3 SNPs (rs10883866, rs10748842 and rs6584400) located in a 13 kb interval in intron 1 of *NRG3*. Our best *p* value from family-based association analysis was $7.26x10^{-7}$. We replicated this association in the collection of 173 unrelated AJ cases ($p = 1.55x10^{-2}$), with a combined *p* value of $2.30x10^{-7}$. With 10,000 permutations of each phenotype, we estimated the empirical study-wide significance across all 9 factors to be $p = 2.7x10^{-3}$. The 20 SNPs with the smallest *p* values in our factor analysis includes a total of 10 (50%) SNPs in *NRG3*, showing association with Delusion and 2 other factors (Scholastic and Disorganization) in different regions of the gene. We also identify 2 novel segregating microdeletions in *NRG3*. *NRG3* is primarily expressed in the central nervous system and is one of 3 paralogs of *NRG1*, a gene strongly implicated in SZ. These biological properties together with our linkage and association results strongly support a model in which multiple alleles of *NRG3* confer susceptibility for different clinical presentations of SZ.

***ATP8B1, ABCB11* and *ABCB4* gene mutation analysis in progressive familial intrahepatic cholestasis patients.** *J. Wang[1], IC. Lee[1,2], ES. Schmitt[1], S. Zhang[1], LC. Wong[1]* 1) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Department of Pediatrics, Chung-Shan Medical University Taichung, Taiwan.

Background: Progressive familial intrahepatic cholestasis (PFIC) is a genetic heterogenous autosomal recessive disorder caused by the defects of bile acids and phospholipids transporters. PFIC is characterized by early-onset progressive cholestasis, and lead to hepatic fibrosis, cirrhosis, and end-stage liver failure before adulthood. There are three loci known to cause PFIC: PFIC1 (*ATP8B1*), PFIC2 (*ABCB11*) and PFIC3 (*ABCB4*). Both PFIC1 and PFIC2 are characterized by normal or near normal serum GGT, while PFIC3 has elevated GGT. PFIC2 results from the defects in canalicular bile salt excretion. PFIC3 is caused by the defects of transporting phospholipid to canalicular lumen. Results: Entire coding exons and adjacent flanking intronic regions of *ATP8B1*, *ABCB11*, and *ABCB4* genes were sequenced. In 360 patients suspected of PFIC, 54 mutations and variants were identified, 34 of them are novel findings. Table 1 summarizes the results of three PFIC gene tests.

PFIC type	Patients	Positives(%)	2 mutations	1 mutation	Mut & Var	Novel
PFIC1	127	14 (11)	8	6	17	11
PFIC2	143	31 (22)	21	10	29	18
PFIC3	90	8 (9)	4	4	8	5

*Conclusions: PFIC is a group of early onset liver disorders with similar clinical phenotypes. We present a spectrum of mutations in ATP8B1, ABCB11, and ABCB4 genes in PFIC patients, with genotype/ phenotype correlations. Mutation analysis is useful for identifying disease etiology and provides information relevant to counseling families.

Specific Sets of Genetic/Genomic Melanoma Changes with Differential Responses to Targeted Therapies. *M. Dalla Palma[1], K.SM Smalley[2], R. Letrero[1], KL. Nathanson[1,3]* 1) Department of Medicine; 2) The Wistar Institute, Philadelphia, PA; 3) Division of Medical Genetics , University of Pennsilvania, Philadelphia, PA.

Understanding the molecular genetics of melanoma provides the basis for the development of rational therapies. To evaluate mutations in oncogenes and tumor suppressor genes involved in melanoma transformation, 78 melanoma primary cell lines were screened for mutations in *BRAF*, *NRAS*, *PTEN*, *CDKN2A*, *TP53*, *CKIT*, and *CDK4*. The identified genetic changes in these cell lines allow us to divide them into groups with distinct genetic profiles. The vast majority (70%) contains *BRAF* mutations, but this large group can be further divided in clusters based on the mutational status of the other genes analyzed. We defined a *PTEN* mutant (32%) and a *PTEN* wild type (17%) group, among which the frequency of TP53 alterations is 24% and 38%, respectively. Another interesting group is represented by six (8%) melanoma harboring both CDK4 and BRAF mutations, all but one in absence of TP53 mutations. Among the melanoma cell lines without BRAF mutations, 12 (13%) carry an alteration in *NRAS*, all at codon 61 and 8 cell lines (10%) are *BRAF* and *NRAS* wild type. Of those, two (2.5%) have *KIT* mutations, two have co-overexpression of *KIT* and *CDK4*, one a *TP53* mutation and three a *CDKN2A* alteration. *CDKN2A* point mutations/deletions (65%) are distributed across the different groups and do not appear any specific sub-type of melanoma. We proved the existence of distinct genomic mutational profiles in melanoma which reflect tumor heterogeneity. Understanding these different mutational groups is essential for the selection of targeted melanoma therapies. Indeed, we have demonstrated that BRAF V600E mutated melanomas with *CDK4* mutation and cyclin D1 amplifications are resistant to *BRAF* inhibitors. Increased resistance to a BRAF-inhibitor was not seen in cell lines with a *CDK4* mutation alone, but was in a cell line with a *CDK4* mutation and *CCND1* amplification. Moreover, we have shown that the group of melanomas and cell lines co-overexpressing CDK4 and KIT are resistant to *BRAF* inhibitors but sensitive to imatinib both in vitro and in vivo using pharmacological studies.

Mitochondrial tRNA Serine Novel Mutation Causes Multi-Systemic Disease. *L. Wong[1], E. Pierce[2], W. Anninger[2], E. Place[3], J. Golden[4], M. Flak[3]* 1) Prof, Dept Molec/Human Gen,MDL, Baylor Col Med, Houston, TX; 2) Dept Ophthalmology, CHOP/UPENN, Philadelphia, PA; 3) Human Genetics, CHOP/UPENN, Philadelphia, PA; 4) Pathology, CHOP/UPENN, Philadelphia, PA.

A mitochondrial etiology should be considered in the face of progressive multi-systemic disease, regardless of initial presenting problems. We report here a 20-year-old Caucasian man with complications of an open neural tube defect involving hydrocephalus, Chiari malformation, bilateral lower-extremity paralysis, bowel/bladder incontinence, scoliosis, restrictive lung disease, osteopenia, and proximal femur/acetabulum deformities. However, a host of multi-system manifestations developed that were not readily attributable to myelomeningocele complications, including: cardiac, CNS, endocrine, ophthalmologic, muscular, and metabolic. Bardet-Biedl GeneChip analysis, common mtDNA point mutations and large deletion screening were negative. Muscle biopsy revealed mitochondrial proliferation, ragged red fibers, scattered COX-negative fibers, coarse granular SDH staining, irregular mitochondrial morphology with lipid deposits. Analyses for respiratory chain complexes and pyruvate dehydrogenase were in the normal range. Leukocyte coenzyme Q content was normal. Sequencing analysis of the whole mitochondrial genome in muscle identified an apparently homoplasmic, novel, 12264 C>T transition in the tRNA serine (AGY) gene. The 12264C is at the last base of the amino acid acceptor stem, crucial for recognition of precursor RNA processing. This mutation disrupts the G-C pair that may be important in the stabilization of tRNA structure and amino acylation. A 12207 G>A transition affecting the same G-C pairing was previously reported in a patient with clinical features of MERRF and MELAS (Wong et al, J Med Genet 2006;43:e46). Both tRNA serine patients had a high percentage of mutant heteroplasmy, mitochondrial proliferation, relative complex I-III deficiency, and hearing loss, whereas only our patient had cardiac involvement and lacked hepatic involvement. This case highlights the value of whole mitochondrial genome sequencing in the diagnostic evaluation of suspected mitochondrial disease.

Recent development of Goldsurfer2 for functional analysis of GWA studies. *F. Pettersson[1], A. P. Morris[1], M. R. Barnes[2], L. R. Cardon[3]* 1) Dept Bioinformatics, Wellcome Trust Centre, Oxford, United Kingdom; 2) Molecular Discovery Informatics, GlaxoSmithKline Pharmaceuticals, Harlow, Essex, UK; 3) Fred Hutchinson Cancer Research Center, Seattle, Washington, USA.

Large-scale systematic genome-wide association (GWA) studies driven by the development of high-throughput genotyping technology seems to have fulfilled its promise for hypothesis generation by identifying a substantial number of highly significantly associated genetic variants for various disorders and phenotypes. In order for the initially promising results to help us increase our understanding of the underlying biological mechanism, in-depth functional characterization needs to be performed. This is likely to involve generation of new large scale dataset from for example resequencing and measuring of gene expression. The Goldsurfer2 (Gs2) software is based around a user-friendly GUI and was developed to be used in GWA studies for data pre-treatment, association testing, together with evaluation, visualization and interpretation of results [1]. The new version of Gs2 has added functionality to handle and analyse sequences, systems biology data such as gene expression and proteomic profiles, and to access functional information from sources such as GO, KEGG and UCSC. A strong emphasis in the recent development is adding support for investigating the link between genetic variants and gene expression (eQTL analysis). Functions for this include a new method for on the fly imputation of genotypes, used in investigating cis effects for nongenotyped variants in candidates. Another new feature identifies variants in microarray probes. With many GWA studies publicly available, it's attractive to combine these for increased statistical power. This can be done in Gs2 even for combining genotypes from different platforms with imputation being instrumental and reference data accessed from a newly designed database. Goldsurfer2 is implemented in Java and is available for Mac OSX, Linux and Windows. [1]Pettersson, F., Morris, A.P.; Barnes, M.R. and Cardon, L.R. Goldsurfer2 (Gs2): A comprehensive tool for the analysis and visualization of genome wide association studies. BMC bioinformatics 2008; 9 (138).

Searching for independent association signals in genome-wide association studies: evidence for a second signal with HDL cholesterol level at the *LIPC* locus. *T. M. Teslovich[1], C. J. Willer[1], L. J. Scott[1], A. U. Jackson[1], L. L. Bonnycastle[2], H. M. Stringham[1], P. S. Chines[2], M. G. Rees[2], T. T. Valle[3], R. N. Bergman[4], J. Tuomilehto[3], F. S. Collins[2], K. L. Mohlke[5], M. Boehnke[1]* 1) U Mich, Ann Arbor, MI; 2) NHGRI, Bethesda, MD; 3) National Public Health Institute, Helsinki, Finland; 4) USC, Los Angeles, CA; 5) UNC, Chapel Hill, NC.

To understand better the allelic architecture of complex traits, we are seeking to identify trait loci at which multiple independent causal variants may be present. For each locus, this will further our understanding of the genetic basis of the trait and may allow better prediction of personalized risk and drug therapies. This knowledge may inform future studies that seek to identify alleles involved in common diseases as the identification of multiple independent signals may provide stronger evidence of true association.

We are examining loci that showed association with lipid levels in a meta-analysis of genome-wide scans performed by the FUSION Study, the SardiNIA Study of Aging, and the Diabetes Genetics Initiative. After follow-up in additional samples, the analysis identified genome-wide significant associations with 18 loci, including one between serum HDL cholesterol levels and SNPs rs261332 (p=2.3x10^{-15}) and rs4775041 (p=3.2x10^{-20}) near LIPC on chr 15. rs261332 is in high LD with promoter polymorphisms that have been associated with HDL. rs4775041 is located ~50kb upstream of LIPC and is not in LD with previously associated variants.

To determine whether rs261332 and rs4775041 represent independent signals, we simultaneously tested both SNPs for association with HDL in 3,738 FUSION samples using linear regression. Neither signal is attenuated in the joint analysis (FUSION p-values: rs261332 unadj 1.2x10^{-7}, adj 3.0x10^{-8}; rs4775041 unadj 1.4x10^{-8}, adj 3.6x10^{-9}). These results suggest that rs4775041 represents an independent association signal at the LIPC locus.

We are currently investigating the remaining loci with preliminary evidence for multiple association signals that may act independently to increase disease risk.

Pseudo Myosin Light Chain Kinase (MLCK) Gene Transcribes in Cancerous Tissues and Cells. *Y. Han[1,2], J. Garcia[2], P. de Lanerolle[1]* 1) Dept Physiology & Biophysics, Univ Illinois, Chicago, Chicago, IL; 2) Dept Medicine, Univ Chicago, Chicago, IL.

Pseudogenes have been defined as nonfunctional genomic sequences that are originally derived from paralogous functional genes. Although biological importance of pseudogenes is not clear, pseudogenes have been widely used as evolution fossils. During the evolution to high primates, a functional MLCK gene was partially duplicated, making a pseudo MLCK gene in humans, chimpanzees, and gorillas. Because MLCK is of critical importance in regulating cytoskeletal dynamics by increasing myosin-actin crossbridge, we investigated the structure and expression of pseudo MLCK gene in humans. A functional MLCK gene, namely MYLK, locates on chromosome 3q21 spanning over 270 kb and contains at least 33 exons that encode 3 proteins: non-muscle MLCK (nmMLCK, 220 kDa), smooth muscle MLCK (smMLCK, 130 kDa) and Telokin (20 kDa). The nmMLCK translates from exon 1 to exon 33 while the smMLCK translates from exon 17 to exon 33. A pseudo MLCK gene, in contrast, locates on chromosome 3p12 and contains only 5 exons which correspond to exons 13-17 of the functional gene. Interestingly, the pseudo gene has a functional promoter for smMLCK at the intron between exons 16-17 and possibly transcribes a part of smMLCK gene. To investigate this possibility, we performed reverse transcriptase PCR (RT-PCR) and Northern blot analyses using RNAs purified from various human tissues with/without cancers. In humans with no diseases, none of tissues or cultured cells expresses the pseudo MLCK. However, it does express mRNA in all of cancerous tissues and cell lines that we have examined so far. These data strongly suggest that the pseudo MLCK gene selectively expresses in the development of cancer. While the functional importance of the pseudo gene expression is to be investigated, this is, to our best knowledge, the first discovery associating a pseudogene expression with a human disease. These data could lead to novel insights into the pathological importance of pseudogenes in the development of human diseases.

Additional Support for the Association of *SLITRK1* var321 and Tourette syndrome. *B. J. O'Roak[1], T. M. Morgan[2], E. Saus[3], P. Alonso[4], M. Gratacòs[3], X. Estivill[3,5], Y. Kohn[6], M. W. State[1]* 1) Dept of Genetics, Program on Neurogenetics, Child Study Ctr, Yale Univ, New Haven, CT; 2) Dept of Human Genetics, WashU, St. Louis, MO; 3) CIBERESP, Genes and Disease Program, CRG, Barcelona, ES; 4) OCD Clinical and Research Unit, Psychiatry Dept, Hospt Universitari de Bellvitge, L'Hospitalet de Llobregat, Barcelona, ES; 5) Experimental and Health Sciences Dept, Pompeu Fabra Univ, Barcelona, ES; 6) Child and Adolescent Psychiatry Unit, Hadassah Hebrew UMC, Jerusalem, IL.

We have identified *SLITRK1* as a candidate for Tourette syndrome (TS) based on the mapping of a *de novo* chr13 inversion; the identification of a frame-shift mutation in the gene in a family with TS and related disorders; and a rare 3 UTR variant (var321) in a miRNA binding domain that was found in 2/174 cases and 0/2148 controls (P=0.0056). The var321 alleles were hypothesized to be of independent origin, further supporting an association (P=0.000056). Subsequent to these initial findings, var321 has been identified at low frequency in affected and control samples (Wendland et al. 2006, Keen-Kim et al. 2006, Scharf et al. 2008). These studies, taken together, demonstrate an overrepresentation in affected individuals. However, two recent publications have proposed that var321 is an Ashkenazi (ASH) allele leading us to misinterpret our original findings. We have examined this hypothesis through extensive genomic characterization of individuals carrying var321 including our previously reported cases (F3/F4), a family with OCD (SP), a human variant panel (HVP) sample and an ASH control (AN). Detailed analysis of the region surrounding var321 supports at least 2 distinct haplotypes (maximal sharing 18kb). Genome-wide SNP data were compared with 1117 White Non-Jewish and 758 Jewish samples using multidimensional scaling analysis. Cases F3, F4 and SP cluster with the Non-Jewish group; HVP and AN cluster with the Jewish group. Finally, Y chromosome and mitochondrial DNA was evaluated in cases F3 and F4 and no ASH-related haplotypes were identified. These data argue strongly against var321 being an ASH variant and support our initial conclusion of an association with TS spectrum disorders.

***NF1* microduplication in a male with atypical café au lait spots.** *M. E. Walker[1], H. M. Saal[1], F. M. Mikhail[2], L. Messiaen[2]* 1) Div Human Genetics, Cincinnati Children's Hospital Med Ctr, Cincinnati, OH; 2) Dept Genetics, Univ Alabama, Birmingham, AL.

Mutations in the *NF1* gene can be identified in ~95% of non-founder patients with neurofibromatosis 1 (NF1) when a full cascade of techniques is applied. Approximately 5% of patients have a 1.4 Mb microdeletion involving *NF1* and typically present with a more severe phenotype. Duplication of the *NF1* gene is apparently rare. We report a *de novo* 1.4 Mb microduplication of *NF1* and flanking genes in a biracial male who presented at age 11 yrs with a prior diagnosis of NF1 based on presence of 6 atypical café au lait spots and hyper-pigmented lesions measuring 0.5-6 cm. MRI at age 9 yrs showed mild distention of the optic nerve sheath suggestive of dural ectasia but no other stigmata of NF1. Clinical exam revealed no axillary/inguinal freckling or skin tumors. He has learning difficulties in several areas. His brother and both parents had normal *NF1* analyses. MLPA analysis of the *NF1* gene in his blood DNA revealed 3 copies of the 1.4 Mb region flanked by REPa and REPc. This duplication was confirmed by interphase FISH probes (RP5-1002G3 & RP5-926B9) that span both ends of the *NF1* gene, demonstrating 3 red/green fusion signals with 2 of these signals co-localizing. The duplication at 17q11.2 is in tandem: metaphase FISH showed an enhanced *NF1* signal on one chromosome 17. Importantly, no mutations were found in the coding regions of the 3 copies of *NF1*. Intragenic microsatellite analysis indicated the duplication was of maternal origin and is heterodisomic, implying a meiosis I error. The only other similar report is a kindred in which an *NF1* microduplication was stable over two generations and carriers were either unaffected or had features of mild MR, early onset baldness, and dental enamel hypoplasia. Our patient may be still too young to present with the latter features. The data do not allow determination of whether the learning problems can be attributed to the microduplication, since his brother without the duplication has mild DD/autism spectrum disorder of unknown cause. More patients must be identified to further understand phenotypes associated with NF1 microduplication.

Association studies of *LOXL1* gene polymorphisms in both high- and normal-pressure Primary Open Angle Glaucoma. *T. Rezaie[1], J. Aragon-Martin[1,2], V. Schmidt[1], L. Beveridge[1], R. Ritch[3], J. Liebmann[3], E. Ilitchev[3], A. Child[2], M. Sarfarazi[1]* 1) Molecular Ophthalmic Genetics Laboratory, University of Connecticut Health Center, Farmington, CT; 2) St. Georges University of London, London, UK; 3) New York Eye and Ear Infirmary, New York, NY.

Primary open angle glaucoma (POAG) is a hereditary optic neuropathy and a common cause of blindness worldwide. In part, POAG is caused by mutations in MYOC, OPTN and WDR36 genes. A recent genome wide study in the Icelandic population identified a significant association between exfoliation glaucoma and *LOXL1* (Lysyl Oxidase-Like 1) gene polymorphisms. Herein, we investigated genetic contribution of 3 coding variants of *LOXL1* in high-pressure POAG and normal-pressure glaucoma (NPG) subjects. All participating glaucoma and normal subjects were Caucasians and received a full ophthalmological examination. A total of 733 (156 POAG, 244 NPG and 333 normal) subjects were genotyped for 3 *LOXL1* variants of R141L (rs1048661), G153D (rs3825942) and S159A. Genotypic and allelic frequencies were tabulated and statistical tests evaluated between normal and glaucoma subjects. The observed R141L allele (G;T) frequencies were: POAG (199;113), NPG (323;165) and controls (464;196). The G153D allele (G;A) frequencies were: POAG (266;46), NPG (413;75) and controls (530;134). The S159A allele (T;G) frequencies were: POAG (311;1), NPG (480;8) and controls (172;8). The S159A was generally uninformative. No significant associations were observed for any of the 3 *LOXL1* coding variations. A marginal allelic (p=0.0363) but not genotypic association (p=0.0732) was observed for G153D in the NPG group. Likewise, when for R141L and G153D the 3 haplotype frequencies of GG, GA and TG (TA was not observed) were tabulated for POAG, NPG and control groups, no significant associations were detected. Our prior studies confirmed that there is a significant association between the *LOXL1* gene polymorphisms for both exfoliation syndrome and exfoliation glaucoma (Mol Vis 2008; 14:533-541). However, present study suggests that no such association exist between *LOXL1* variations with both high- and normal-pressure glaucoma. Supported by M01RR-06192.

Combining pedigree and high-density markers for genome-wide association studies. *D. Heckerman[1], C. Kadie[1], H. M. Kang[2]* 1) Microsoft Research, Redmond, CA; 2) Department of Computer Science and Engineering, University of California, San Diego, California 92093.

In genome-wide association mapping, family-based designs are known to be robust to confounding due to (e.g.) population structure. Unfortunately, these studies often suffer from lack of power because they ignore the relationships among families, which is often impossible to obtain. The recent availability of high-density genotype data not only improves the genomic coverage of disease loci, but also enables accurate estimation of genetic relatedness between individuals. Consequently, we propose an approach that combines pedigree and high-density markers for genome-wide association studies. The approach uses a linear mixed model for quantitative phenotypes or a generalized linear mixed model (GLMM) for binary phenotypes wherein the random effects are modeled by a directed acyclic graphical (DAG) model (equivalent to a multivariate-Gaussian) that combines pedigree and marker information. In particular, known relationships in the pedigree are translated to the graphical model in the usual way (e.g., Wright 1922) and the remaining relationships are inferred from the markers (e.g., via estimates of IBD or IBS coefficients). A variational approximation is used to estimate the parameters of the GLMM, yielding a novel approach that provides a conservative estimate of likelihood ratio statistics that is efficient to compute. Our investigations with simulated and real data including that from GAW14 demonstrate that the approach robustly corrects for inflated false positives due to population structure and has increased power over family-based designs.

Mitochondrial Genetics of Outcomes Attained after Traumatic Brain Injury. *Y. Conley, S. Alexander, S. Deslouches, D. Ren* University of Pittsburgh.

Traumatic brain injury (TBI) results in a cascade of events at the cellular level that have the potential to cause secondary injury. A better understanding of the mechanisms of secondary injury could result in improved intervention at a time when further injury can be avoided and long term outcomes following TBI optimized. The purpose of this study is to determine if the mitochondrial genome and mitochondrial function account for secondary injury and is associated with variation in long term outcomes. Outcomes are defined by Glasgow Outcome Scale (GOS) following severe TBI, defined as a Glasgow Coma Score (GCS) of 8 or below. To address this, cerebrospinal fluid (CSF) drained as standard of care from TBI victims for the five days following injury is evaluated for mtDNA integrity, mtDNA level and ATP level. A subset of our available subjects have been evaluated to date (n=111) for level of mtDNA; measured using qRT-PCR and normalized with simultaneous qRT-PCR for betaglobin; and level of ATP using a luciferase based assay. MtDNA and ATP level for the first five days post injury were not impacted by age, gender or GCS, which are well known potential confounders when assessing outcomes following TBI. While mtDNA level did not significantly change over the first five days after injury, ATP level approached significance (p=.0630). GOS at 3, 6, 12 and 24 months was not significantly associated with mtDNA level. This preliminary data potentially indicates that quality of mtDNA and not mtDNA quantity may be important during the acute period following TBI given that mtDNA quantity does not change considerably during the acute period, however it appears that ATP production does. This is the first study to investigate mtDNA quantity during the acute period following TBI and long term functional outcomes and from this data it does not appear that mtDNA quantity plays a role in long term functional outcomes after TBI.

A comprehensive gene network based on genetic correlations among gene expression levels. *H. H. H. Göring, E. I. Drigalenko, J. E. Curran, T. D. Dyer, J. C. Charlesworth, M. P. Johnson, S. E. Cole, E. K. Moses, J. Blangero* Dept. of Genetics, Southwest Foundation of Biomedical Research, San Antonio, TX.

Differences in gene expression levels are thought to be important contributors to risk of complex disease. By now it is feasible, using commercial technologies, to quantify expression levels of the entire human transcriptome. Recently, multiple large-scale investigations have mapped the genetic determinants influencing the expression levels of individual transcripts. We have previously reported on genetic analyses of expression profiles in lymphocytes from 1,240 Mexican American participants in the San Antonio Family Heart Study. We, and others, have demonstrated the frequency and strength of proximal genetic effects (presumed to act in *cis*) on gene expression regulation. Conversely, distal genetic factors (presumed to act also in *trans*) were found less frequently and exhibit weaker effects. Given the scarcity of distal (*trans*) signals, it is difficult to build gene networks from such data. To overcome this challenge, we have computed (using ~40 computer processor years) all pairwise phenotypic, genetic, and environmental correlations among the 11,493 autosomal RefSeq transcripts that we detected and whose quantitative expression levels were significantly heritable at an FDR of 1%. Using bivariate variance components analysis, we obtained the genetic and environmental correlations for these >66 millions pairs of transcripts based on the pattern of covariation among relatives in our extended pedigree sample. Formal tests of our empirical genetic network yield evidence for intergenic relationships that is consistent with other classical sources of information (such as physical interaction data provided by 2-hybrid systems). We plan to make this resource freely available via the web, enabling researchers to rapidly build empirical gene networks by identifying those genes whose expression levels are significantly genetically correlated with that of a gene (or multiple genes) of interest. Genetically-correlated genes represent good candidates---chosen by an objective criterion---to act upstream, downstream or in parallel to the gene(s) of interest.

Genotype-by-sex interactions influence the regulation of human gene expression. *L. E. Bauman, J. C. Charlesworth, J. W. Kent, Jr., J. E. Curran, M. P. Johnson, S. E. Cole, T. D. Dyer, E. K. Moses, J. Blangero, H. H. H. Göring* Southwest Foundation for Biomedical Research, San Antonio, TX.

Men and women differ from one another biologically in many ways, including life span, risk of various diseases and response to drugs. To identify sub-cellular differences between males and females, we performed a comprehensive evaluation of the importance of sex effects on gene expression levels, including the detection of genotype-by-sex (GxS) interaction. Genome-wide expression profiles were previously generated on lymphocytes from 1,240 Mexican American participants in the San Antonio Family Heart Study (506 males and 734 females). The current investigation focused on 12086 autosomal RefSeq transcripts whose expression level was heritable at an FDR of 5%, used as the significance criterion throughout this study. Using a mixed model approach, we found that nearly 1/3 of all transcripts (3935) vary significantly in gene expression level between sexes, with 43% showing higher expression levels in females and 57% in men. Next, we assessed whether the genetic architecture underlying the expression of individual transcripts varies between the sexes by modeling additive polygenic GxS interaction, and found significant interaction for almost 10% of transcripts (1154). Interestingly, over 99% of these transcripts have higher genetic variance in females than in males. Finally, we considered whether particular locations in the genome differentially influence gene expression levels among males and females. Using a linkage-based GxS interaction model and focusing on those transcripts with significant evidence of proximal (and likely cis) genetic regulation, we discovered GxS interaction at the position of the structural locus for 57 genes. We are currently in the process of identifying the specific genetic variants responsible for the observed GxS interaction within these genes. Our results highlight the general importance of sex effects on transcription and the substantial role that GxS interactions play in the regulation of human gene expression.

Analysis of neuregulin 1 missense mutations in Costa Rican families. *A. Davelos Baines[1,4], C. Walss-Bass[1,2], A. Figueroa[1,5], R. Salazar[3], A. Dassori[2], J. Peters[2], I. Chavarria-Siles[2], H. Loria[4], J. Cardoza[4], M. Escamilla[1,2], H. Raventos[3]* 1) South Texas Medical Genetics Group, University of Texas Health Science Center San Antonio, Edinburg, TX; 2) Department of Psychiatry, University of Texas Health Science Center, San Antonio, TX; 3) Centro de Investigacion en Biologia Celular y Molecular, Universidad de Costa Rica, San Pedro, Costa Rica; 4) Department of Biology, The University of Texas - Pan American, Edinburg, TX; 5) Department of Computer Science, The University of Texas - Pan American, Edinburg, TX.

Background: A missense mutation (Val to Leu) in the transmembrane domain of the neuregulin 1 (NRG1) gene has been associated with schizophrenia in a population from the Central Valley of Costa Rica (CVCR). Missense mutations in exon 2 (rs3924999) and exon 12 (rs10503929) of NRG1 have been previously identified but had not been examined in this population. Methods: DNA genotyping and association studies were performed for 442 individuals with psychosis (243 of whom had a diagnosis of schizophrenia) and their relatives (1167 subjects total) from the CVCR. Transmission disequilibrium tests were performed using Family Based Association Tests (FBAT). Results: Association analysis revealed that the exon 12 missense mutation was significantly associated with psychosis ($p = 0.010$), and with schizophrenia ($p = 0.04$), but after correcting for multiple testing, only the association with psychosis remains significant. There was no association of the exon 2 missense mutation with either psychosis ($p = 0.38$) or schizophrenia ($p = 0.59$). Discussion: This study reveals significant association of a missense mutation in exon 12 of NRG1 with psychosis. Further investigation of functional differences among variants is required to identify the basis for this association.

Structural variation classification and detection based on paired-end read patterns of coverage. *C. Stewart, G. Marth* Biology, Boston College, Chestnut Hill, MA 02467.

The advent of next-generation sequencing technologies using paired-end reads allows for the detection of structural variations at unprecedented levels of efficiency and precision. Here we describe a classification scheme for 9 fundamental types of structural variants based on distinct patterns of paired-end read genome coverage. The SV types are: 1. deletion, 2. tandem duplication, 3. dispersed duplication, 4. VNTR, 5. novel insertion, 6. repeat insertion, 7. local translocation, 8. inter-chromosomal translocation, 9. inversion. Classification and measurement accuracy depends on the choice of technology as well as parameters such as the read length, depth of coverage, fragment length, and the dispersion of the fragment length distribution. Simulated sequence data is used to assess detection accuracy for each type of structural variant for typical choices of sequencing parameters. The algorithms performance is also assessed with human paired-end read data from the Illumina, AB, and 454 platforms. A given paired-end read alignment is quantified in terms of the genomic position of the leading read (p), the distance between the aligned ends of the fragment (LM), the orientation of the read pairs (o), as well as the confidence that each end is uniquely aligned. The number of reads overlapping a given base in the reference genome is the depth of coverage (d). Each of the 9 structural variant types has a distinct pattern in (p,LM,o,d) space which is used as a constraint in the classification algorithm. For example, deletion events are characterized by a cluster of fragments with exceptionally long LM spanning a region in the reference genome with low depth of coverage. A chromosomal translocation event is identified as a cluster of fragments with long LM spanning a region of normal depth of coverage adjacent to clusters of read pairs that span across regions on two chromosomes. The algorithm is implemented as a command-line tool publicly available at http://bioinformatics.bc.edu/marthlab/software/Spanner. A visualization tool is also under development as an aid for researchers to scan and assess evidence for candidate structural variant events.

Novel duplication in the PDHA1 gene in a family with 3 variably affected females with pyruvate dehydrogenase deficiency. *C. R. Coughlin[1], F. Y. Li[2], L. J. Wong[2], J. Ganesh[1]* 1) Biochemical Genetics, Children's Hospital of Philadelphia, Philadelphia, PA; 2) Baylor College of Medicine.

Pyruvate dehydrogenase complex (PDHC) deficiency is a clinically heterogeneous disorder with symptoms ranging from asymptomatic carrier status, chronic neurological dysfunction of varying degree or fatal neonatal lactic acidosis. The majority of cases are due to mutations in the X - linked, E1 subunit gene (PDHA1). Insertions/deletion mutations especially in Exons 10 or 11 are common in females and missense mutations predominate in males. A 10 month old girl was referred for global delay, lactic acidosis, microcephaly, WPW syndrome and seizures. MRI of the brain revealed agenesis of the corpus callosum. Activities of PDHC and respiratory chain complexes were normal in skeletal muscle. As clinical features and biochemistry were suspicious for PDHC deficiency and the delivery of a sibling was imminent, molecular studies were undertaken. Sequencing of the E1 alpha subunit gene revealed a novel 26 nucleotide duplication within exon 10 (c.900-3_c.922dup). The duplication creates a frameshift and premature termination codon which is predicted to be deleterious. PDHC activity was subsequently confirmed to be low in skin fibroblasts. A female sibling was born shortly after the diagnosis was confirmed and presented with microcephaly, growth retardation, hypotonia and high blood lactate and pyruvate levels. Brain MRI done at 2 weeks of age revealed periventricular leukomalacia, but her overall clinical picture was milder than the proband. Molecular testing revealed the neonate had inherited the familial duplication. Ketogenic diet was started at 6 weeks of life and at the age of 7 months, she is thriving and gaining milestones. Parental testing revealed that the mother who has mild microcephaly and learning disability was heterozygous for the duplication. A novel mutation in PDHA1 variably affecting multiple generations within a family is described. This case underscores the value of molecular testing for PDHA1 mutations even if PDHC activity is normal in peripheral tissues especially in females. This data has significant impact on counseling and management of PDHC deficiency.

Characterization of gene expression in severe forms of dengue virus infection. *M. Yasunami[1], N. T. P. Lan[1], M. Kikuchi[1], V. T. Q. Huong[2], V. T. T. Ngu[2], H. N. Dao[2], D. Q. Ha[2], T. T. Thuy[3], H. M. Tuan[3], K. Morita[1], K. Hirayama[1]*
1) Inst Trop Med, Nagasaki Univ, Nagasaki, Japan; 2) Pasteur Institute in Ho Chi Minh City, Ho Chi Minh City, Vietnam; 3) Nhi Dong Hospital No.2, Ho Chi Minh City, Vietnam.

Dengue fever is caused by acute infection by dengue virus. Severe illness designated as dengue hemorrhagic fever (DHF) develops in small part of the patients, which is recognized by hemorrhagic tendency and plasma leakage from blood vessels in addition to general febrile symptoms. Among the patient with DHF, severe plasma loss causes hypovolemic shock (dengue shock syndrome, DSS) which requires further intensive medical care. Excessive host response known as cytokine storm has been reported to accompany to these severe cases, but pathogenesis of severe illness remains to be elucidated. For the better understanding of host determinants for disease severity, RNA expression profiling was conducted in the present study. Ten children aged from nine months to 14 years who admitted to Nhi Dong Hospital No.2 in Ho Chi Minh City, Vietnam in August and November, 2007 because of clinical symptoms meeting WHO criteria of DHF grade II (corresponding to DHF, 6 cases) or grade III (corresponding to DSS, 4 cases) were enrolled. In addition to routine hematological and virological examinations during hospitalization, paired blood samplings for RNA preparation were done on the day of onset of severe illness and several days later when the most symptoms disappeared. Ten pairs of RNA samples were then analyzed for the levels of 48,701 transcripts by Illumina Human-6 Expression Bead Array. Seven genes including complement component 2 (*C2*) were expressed more than two-fold and nine including genes for Protein S (*PROS*) and von Willebrand factor (*VWF*) exhibited less than half on the day of onset in comparison to the recovery phase in all ten pair comparisons. The results suggested the dysregulation in blood coagulation and complement system is common background for hemorrhagic manifestations. Further analyses of these data will provide certain insights for determinants of disease severity between DHF (grade II) and DSS (grade III).

Comprehensive mutational analysis of BRCA1/2 using direct sequencing and gene dosage analysis. *H. Park[1], M. Seong[1,2], S. Cho[1], D. Noh[3], W. Han[3], S. Kim[4], C. Park[1], S. Kim[1], S. Park[1]* 1) Department of Laboratory Medicine, Seoul National University Hospital & Clinical Research Institute, Seoul, Korea; 2) Department of Laboratory Medicine, National Cancer Center, Goyang, Korea; 3) Department of Surgery, Seoul National University Hospital, Seoul, Korea; 4) Department of Surgery, Seoul National University Bundang Hospital, Seongnam, Korea.

Purpose: The BRCA1 and BRCA2 genes are the strongest susceptibility genes of breast cancer worldwide. However, BRCA1/BRCA2 would be incompletely investigated due to their large size and genomic rearrangement occasionally involving them. Here we performed comprehensive mutational analysis for BRCA1/BRCA2 in 206 Koreans. Methods: We analyzed all exons and flanking regions of BRCA1/BRCA2 by direct sequencing and screened deletions or duplications involving BRCA1/BRCA2 by multiplex ligation-dependent probe amplification. We constructed haplotypes using several intragenic SNPs with minor allele frequency threshold of 5% to investigate founder effect of recurrent mutations and genotype-phenotype association. Results: In our series, thirty-eight patients (18.4%) including a double heterozygote had one or more BRCA1/BRCA2 mutations. Four additional patients carried distinct unclassified variants of possibly harmful effect. Any large deletion or duplication involving BRCA1/BRCA2 was not identified in our series. Haplotype analysis suggested that the most frequent mutation BRCA2:c.7708C>T might be originated from a common ancestor. We confirmed that this mutation was in a tight linkage with the specific haplotype using allele separation in some patients. BRCA1/BRCA2 mutations were more frequent in a group with family history, bilateral cancer, or multiple site cancer than group without the risk factor concerned or unknown risk group. In contrast, mutation frequency in early-onset cancer group was similar to unknown risk group. Conclusions: This is the first comprehensive mutation report using both direct sequencing and dosage analysis in the Korean population, and the first report showing possibility of a founder mutation in this population.

Detecting Essential Interactions in Genome-Wide Association Studies. *C. Wu[1], H. Zhang[4], X. Liu[2], Z. Ying[3], Y. Yang[4], J. Hoh[1]* 1) Epidemiology and Public Health, Yale University, New Haven, CT; 2) Department of Applied Mathematics, Yale University, New Haven, CT; 3) Department of Statistics, Columbia University, New York, NY; 4) Department of Statistics and Finance, University of Science and Technology of China.

Gene-gene interactions have become the next threshold in the analysis of the genome-wide association (GWA) data now that single-gene main effects are being found at an accelerating pace. A typical GWA dataset now produces ½ million to almost 2 million genetic markers. Many of these markers have some level of dependence on nearby markers owing to linkage disequilibrium (LD). As such, exhaustive testing of the astronomical number of interactions in this classic Np-hard problem is computationally infeasible for any GWA study. Statistical interactions are characterized into two distinct classes, the essential interactions and the removable interactions. Essential interactions (EIs) are invariant under any risk measures while removable interactions (RIs) are sensitive to the choice of the risk functions that measure the effects. In light of the need to reduce complexity in the GWA data, we propose a fast and reliable screening strategy based on the necessary and sufficient condition for an interaction to be essential. The likelihood ratio test and a resampling procedure are developed to evaluate statistical significance of the EIs. Simulation analyses show the proposed approach having sufficient statistical power with the false-positive error rates being small and tolerable. This approach has been applied to a real GWA datasets.

Preimplantation Microarray Analysis (PMA) is a Robust technique that allows for Aneuploidy Screening of all 24 chromosomes with a lower Misdiagnosis rate than FISH based methodologies. *B. Levy[1,2], O. Nahum[1], M. Kamani[2], K. Miller[2], J. Su[2], N. Treff[2], R. Scott, Jr.[2]* 1) Dept Pathology, Columbia University, New York, NY; 2) Reproductive Medicine Associates of New Jersey, Morristown, NJ.

The blend of whole-genome amplification & microarray technology may provide an attractive solution to the limitations of current preimplantation FISH-based aneuploidy screening strategies. We have previously validated the use of SNP oligonucleotide microarray analysis (SOMA) for the detection of aneuploidy in IVF blastomeres. In order to demonstrate the accuracy of SOMA, we compared the consistency of the results in embryos analyzed both by a 9 chromosome FISH panel & by SOMA. 8 arrested cleavage stage embryos were biopsied into individual cells, randomized into even groups & blindly assigned for further analysis by FISH (n=51) or SOMA (n=52). 6/8 embryos showed a consistent diagnosis for all cells analyzed (n=39) by SOMA. The remaining 2 embryos showed mosaicism that made biological sense (i.e. 46,XY; 45,XY,-13; 47,XY,+13). Every embryo analyzed by FISH showed mosaicism with little consistency in the diagnosis between cells derived from the same embryo. The robust nature of PMA was further validated by comparing the consistency of the results obtained from cells analyzed both by CGH and SOMA. DNA aliquots from 10 coded blastomeres were subjected to CGH and SOMA. The sex matched perfectly for all cells. The copy number for each chromosome was concordant in 97.5% of the 240 chromosomes analyzed. The FISH results of 4304 embryos were also compared to the SOMA results from 505 embryos. Using the SOMA data as a standard, misdiagnosis rates were calculated by contrasting the overall abnormality rate per embryo in embryos diagnosed by FISH vs SOMA. FISH (9 chromosome panel) had an overdiagnosis rate (dx as abnormal when embryo is actually normal) of 40% & an underdiagnosis rate (failure to identify abnormals in chromosomes not tested) of 12%. These studies indicate that PMA assays likely offer a superior means for aneuploidy screening in IVF embryos compared with FISH. They may also partially explain the failure of FISH PGD to produce meaningful improvements in clinical outcomes for IVF patients.

MESA-A Novel Method to Detect Copy Number Polymorphisms. *B. Zhang, J. Li, J. Andriesen, J. Hanson, L. Zhao*
Fred Hutchinson Cancer Research Center, Seattle, WA.

Copy number polymorphisms (CNP) have been discovered in the human genome, and associations have been seen between CNPs and complex phenotypes. The recent development of array technologies with probes specifically designed to detect CNP sites has further stimulated interest in discovering disease-associated CNP in the human genome. To enable these studies, detecting and cataloging CNP within the study population is critically important. Here we propose a new statistical method for detecting CNP sites using genome-wide SNP array data. The method utilizes moving windows throughout the genome to estimate CNP segments and their phenotypic associations. We term this method MESA (moving estimation of segmentation and associations). When MESA is applying to data from Affymetrix Genome-Wide Human SNP 5.0 and 6.0 arrays, it utilizes intensity signals from all probes, including all CN probes and the multiple probes for individual SNP alleles. The method computes the log ratios of probe intensity values over those from a reference population. Log ratios are then used for detecting segmentation and smoothing signals within each segment for every individual. To detect CNP sites in our study population, MESA repeats the segmentation process on the population-averaged signals from smoothed data. Gaussian mixture model is used to detect the presence of multiple modes in the log ratio values, the confirmation from which leads to the assignment of CNP sites. MESA was used on data generated from 3000 samples with the Affymetrix SNP Array 5.0. A total of 130 CNP sites were identified in this population. To indirectly validate these discoveries and hence the method, we compared our identified CNP sites with those discovered using other methods. Our findings overlapped with sites identified in other studies while also identifying several potentially novel CNP sites in our population. While our results remain preliminary, it suggests that MESA is robust and efficient for the detection of CNP sites. Due to its ability to utilize information from differing probe types, MESA is readily applicable to genomewide data generated by other arrays or other technology platforms.

Fabry Disease: 11 Novel -Galactosidase A Mutations. *R. Dobrovolny[1], D. Kwan[1], M. Rudelli[1], S. Garman[2], I. Nazarenko[1], M. Yasuda[1], R. J. Desnick[1]* 1) Department of Genetics & Genomic Sciences, Mount Sinai School of Medicine, New York, NY; 2) Department of Biochemistry and Molecular Biology, University of Massachusetts, Amherst, MA.

Fabry disease is an X-linked glycosphingolipidosis resulting from the deficiency of lysosomal exoglycosidase, -galactosidase A (-Gal A). To date, over 500 disease causing mutations have been reported. Most are family specific and only a few are more frequent (e.g. R122H, A143T, N215S, R227X). Incidence of the Fabry disease is about 1:40000 males but it was shown that many of the patients remain undiagnosed. We report 11 previously undescribed mutations in the -Gal A gene (GLA) causing the classic phenotype in unrelated families. These include six missense mutations (M1K, Y86D, W95L, G183V, L275F, L372R), four nonsense mutations (E7X, K82X, E103X, W287X) and one small deletion (c.883delT). Of the missense mutations, M1K occurred in the initiation codon and completely blocked the translation of active enzyme. Mutations Y86D, W95L and G183V occurred in highly conserved residues which were buried in the enzymes 3D structure of catalytic domain and were predicted to cause instable enzyme polypeptide. Change of leucine 372 for arginine in L372R mutation leads to steric clash in noncatalitic domain of the molecule and predicts instability of the enzyme structure. Though not highly conserved, in all plant and animal -galactosidases as well as in phylogeneticaly related -N-acetylgalactosaminidases this position was occupied by a small residue such as leucine, isoleucine or glycine. The dysfunction of the missence mutations was also confirmed by in vitro expression assays. These studies further define the molecular heterogeneity of the -Gal A mutations in Fabry disease and provide insight into -Gal A structure-function relationships.

EMINIM : An accurate, rapid, and memory efficient method for imputation of unobserved genotypes. *H. M. Kang[1], N. A. Zaitlen[2], B. Han[1], E. Eskin[3,4]* 1) Computer Sci Engineering, Univ California, San Diego, La Jolla, CA; 2) Bioinformatics Program, Univ California, San Diego, La Jolla, CA; 3) Department of Human Genetics, University of California, Los Angeles, Los Angeles, CA; 4) Department of Computer Science, University of California, Los Angeles, Los Angeles, CA.

The availability of comprehensive reference panel for genetic variation map such as HapMap enables us to increase the coverage of genome wide association studies by inferring unobserved genotypes from the genotypes collected through high-density genotyping arrays. We propose a novel imputation method EMINIM for accurate, rapid, and memory efficient genotype imputation. Our method learns the parameters in the hidden Markov model from the genotype data with an exact EM algorithm rather than using predefined parameters or simplfying the model. Our method is several times faster than previous method by reducing the time complexity from quadratic to linear time with respect to the number of states. Our method also consumes an order of magnitude less memory by maintaining the states only for observed genotypes. We applied our method to the imputation of genotypes in the inbred mouse strains, and achieved imputation error of 0.37% in high-confidence category, which is more ten times smaller than the previous studies. Our application of EMINIM to Wellcome Trust Case Control Consortium (WTCCC) dataset also shows significant improvement in the imputation accuracy as well as dramatic reduction both in the computational cost and the memory usage over the previous methods.

Deletion of Tumor Necrosis Factor-alpha delays neurodegeneration in Sandhoff mice. *H. Abou-Ouf[1], M. Melas[1], A. Fiebig[1], B. Trigatti[3], S. Igdoura[1,2]* 1) Biol Dept, McMaster Univ, Hamilton, ON, Canada; 2) Department of Pathology and Molecular Medicine, McMaster University, Hamilton, ON, Canada; 3) Department of Biochemistry and Biomedical Sciences, McMaster University, Hamilton, ON, Canada.

Neuro-inflammation is considered a prominent pathogenic feature in several neurodegenerative diseases. Tumor necrosis factor-alpha (TNF-) has been demonstrated as a key modulator of the CNS immune response. Elevated levels of TNF- have been detected in Alzheimers disease, Parkinsons disease, HIV-dementia, G_{M2} gangliosidosis and other neurodegenerative diseases. However, TNF- has dual, neurodegenerative and neuroprotective functions as well as differential region-specific effects. A variable net effect of TNF- on the pathogenic process may be attributed to the profile of microglial distribution and their inflammatory mediators and as such the overall impact of TNF- has been difficult to decipher. In our studies, the involvement of TNF- in the neurodegenerative process was evaluated using a mouse model of Sandhoff disease. Sandhoff disease is a lysosomal storage disorder caused by deficiency in hexosaminidase B (hexb). We generated a double knockout of TNF- and hexb and compared the resulting phenotype with single knockout mice. Our behavioral analysis revealed that Sandhoff mice without TNF- showed an ameliorated disease course. The double knockout mice showed prolonged lifespan and reduced weight loss when compared to hexb knockout. Furthermore, removal of TNF- from Sandhoff mice improved their performance in several neurological tests such as wire hang, rotorod and righting response tests. Using thin layer chromatography, we found no discernible difference in the levels of accumulated G_{M2} and G_{A2} in the cerebral cortex and cerebellum. We propose that neurological improvement was achieved due to reduced neuro-inflammatory condition rather than localized correction of neuronal storage. In conclusion, our results reveal TNF- as a broadly relevant cytokine in the progression of neurodegeneration and as a potential therapeutic target to attenuate neuro-pathogenesis.

Whole Genome Association Analysis of Dynamic Complex Traits. *R. M. Salem[1], E. N. Smith[2], S. S. Murray[2], G. S. Berenson[3], D. T. OConnor[1], N. J. Schork[1,2]* 1) Univ California, San Diego, La Jolla, CA; 2) Scripps Genomic Medicine, TSRI, La Jolla, CA; 3) Tulane University, New Orleans, LA.

Recent advances in human genetics and genotyping technologies have ushered a paradigm shift in genetic research from family based to whole-genome association (WGA), population based studies. This shift in emphasis has allowed researchers to probe the genetic basis of common complex traits in specific ways. Unfortunately, recent WGA studies have provided few insights into the genetic basis of many common complex diseases, identifying loci that explain only a small fraction of the variation. This is in part due to the complexity of the diseases themselves, but also due in part to study design issues. WGA studies have narrowly emphasized the case-control design. The case-control study design is an efficient and powerful approach to study disease risk factors. However, in case-control studies, complex traits are simplified, which typically have underlying quantitative liabilities, to a binary outcome (e.g., presence vs. absence). In reality, the study of complex traits is not always so simple nor are such simple strategies always effective. Instead, genetic researchers should move to studies of dynamic complex traits (DCTs), that use longitudinal or temporal information in, for example, prospective cohort designs. The study and analysis of DCTs in longitudinal contexts can offer insights into disease pathogenesis that are not achievable in other study designs and research settings. Statistical methods for the assessment of DCTs are challenging, and many are known to be inappropriate or perform poorly or do not fully utilize all available data. Height data from the Bogalusa Heart Study, a long-term study concerned with the early natural history of CVD of children aged 5 to 17, is used to illustrate these issues. Strategies for efficient WGA analysis of DCTs, including both novel and traditional analysis are developed and explored, and areas that demand further consideration are presented. Ultimately, this work has the potential to both shed light on the most appropriate approach to the WGA analysis of DCTs and help promote the use of DCTs in genetics research.

The Chromosome: What is it? *K. M. Polacek[1,2], J. C. Walz[2], S. L. Elrod[2]* 1) Fielding Graduate University, Santa Barbara, CA; 2) California Polytechnic State University, San Luis Obispo, CA.

To the learner, the language of genetics is filled with complicated terminology and jargon that experts take for granted. To successfully learn major genetics concepts, students must master these idiomatic and often confusing terms. Specifically, research has shown that students have difficulty fully understanding the nature of chromosomes, which are arguably an essential genetic concept. For example, students may believe that a condensed metaphase chromosome is a pair of chromosomes. Given that textbooks are a common reference source for students, we reviewed 6 current texts for the words and concepts found in various textbook features used to describe the term chromosome. Our preliminary analysis revealed significant inconsistencies and deficiencies across the textbooks. Glossary definitions varied in several ways: one did not include the fact that chromosomes contain DNA; another did not include the term chromosome; one text did not have a glossary at all. The indexes contained between 20-101 headings and sub-headings for the term chromosome, frequently with no page number indicated for the actual term. Even when a page number was indicated, the definition found on that page was not descriptive; one text defined chromosomes as discrete structures of DNA. Additionally, figures of chromosomes included photomicrographs and drawings of replicated, unreplicated, condensed, and uncondensed chromosomes; and, there was variation in the inclusion of terms such as allele, sister chromatid, and homologous chromosomes in associated figure captions. Our in-depth analysis of textbook features used to describe the chromosome and related terms (i.e., chromatid) will be presented. We will also suggest ways in which textbook features might be modified to enhance both the teaching and learning of genetics. As a ubiquitous teaching tool, the textbook is an ideal place to begin addressing fundamental misunderstandings in genetics. This analysis, in combination with investigations into student understanding using concept inventories and other authentic assessment measures, will contribute to the improved student learning of important genetic concepts.

Identification of cytogenetic abnormalities in prenatal specimens by high-resolution microarray. *L. G. Shaffer, S. Alliman, J. Coppinger, B. A. Bejjani, B. A. Torchia* Signature Genomic Laboratories, Spokane, WA.

Microarray-based comparative genomic hybridization (aCGH) is a powerful genetic testing tool for the detection of chromosome abnormalities in individuals with mental retardation and congenital anomalies. However, few prospective prenatal studies have been reported. We performed high-resolution aCGH using either a bacterial artificial chromosome (BAC) or oligonucleotide microarray on 138 prenatal samples received between November 2007 and April 2008. Of these 138 samples, 85 were referred for abnormal ultrasound; clinically significant results were identified in five of these cases, and unclear results requiring testing of parental samples for full interpretation were reported in five. By subsequent parental testing and analysis of gene content and CNV databases, all five unclear results were interpreted as benign CNVs. In 53 samples referred for AMA, family history, or parental concerns, four benign CNVs were detected. The detection rate of clinically significant chromosome abnormalities in our prenatal population (3.6%) is lower than that reported for postnatal specimens. However, these results show a substantial increase in the detection rate compared to our recently reported yield of 1.1% (2/151) in prenatal specimens analyzed using a targeted microarray. In both study populations, all prenatal samples had previous normal karyotypes; samples with known abnormal karyotypes were excluded. These results suggest the yield of chromosome abnormalities detected by microarray analysis would be higher in a prenatal population not already tested by karyotyping.

Clinical overlap between Joubert and Pallister-Hall syndromes suggests a role for ciliary proteins in sonic hedgehog signaling pathways. *M. Parisi, D. Doherty, D. Knutzen, P. Chance, I. Glass* Dept Ped/Div Gen & Develop, Children's Hosp & Reg Med Ctr, Seattle, WA.

Pallister-Hall syndrome (PHS) is an autosomal dominant disorder characterized by polydactyly, hypothalamic hamartomas, bifid epiglottis, imperforate anus, pituitary insufficiency, mental retardation, and renal abnormalities. Mutations in GLI3, a gene important in the Sonic Hedgehog (SHH) pathway, are identified in ~95% of affected individuals. Although hypothalamic hamartomas are considered diagnostic, epiglottic abnormalities are also a useful sign. Joubert syndrome and related disorders (JSRD) are autosomal recessive ciliary conditions with a distinctive cerebellar malformation known as the "molar tooth sign" (MTS), hypotonia, mental retardation, apnea/tachypnea, and oculomotor apraxia. Polydactyly, oral frenulae, renal cystic disease, and encephaloceles are variable findings. We report two cases with overlap between PHS and JSRD. Case 1 was a male infant with postaxial polydactyly of the hands and bifid great toes. He had midline upper lip and tongue grooves, redundant oral frenulae, and an abnormal epiglottis with midline cleft. A diagnosis of PHS was considered, but no causative GLI3 mutation was found. An MRI repeated at 2 years showed a MTS, consistent with JSRD. JSRD genetic analysis is pending. Case 2 was a male with coarctation of the aorta, bifid epiglottis, postaxial polydactyly of the hands, and preaxial polydactyly of the feet. He developed growth hormone deficiency. MRI scan was normal as was GLI3 analysis. Repeat MRI scan for an expanding nasal dermoid cyst showed the MTS. Testing for mutations in 4 JSRD genes was normal. These two cases have epiglottic abnormalities and polydactyly consistent with PHS, but cerebellar findings of the MTS. Thus far, no genetic cause has been identified, but they most likely have recessive mutations in a JSRD gene. The overlap between PHS and JSRD suggests that the primary cilium plays a role in SHH signaling pathways, as has been shown in mouse models. Thus, ciliary genes may affect GLI3 expression and developmental pathways that determine midline hindbrain, laryngeal, and craniofacial structures and polydactyly.

Stochastic DNA methylation of mouse retrotransposons depends on age of the insertion. *D. L. Mager[1,2], M. Reuter[3], Y. Zhang[1,2], D. Reiss[1,2]* 1) Terry Fox Laboratory, BC Cancer Research Centre, Vancouver, BC, Canada; 2) Dept. of Medical Genetics, University of BC; 3) Research Dept for Genetics, Evolution and Environment, University College London, United Kingdom.

Phenotypic variation stems from both genetic and epigenetic differences between individuals. To elucidate how phenotypes are determined, it is necessary to understand the forces that generate variation in genome sequence as well as in its epigenetic state. In both contexts, transposable elements (TEs) may play an important role. It is well established that TE activity generates genetic variation, but recent research also suggests that TEs contribute to epigenetic variation. Stochastic epigenetic silencing of some TE insertions in mice has been shown to cause phenotypic variability between individuals. However, the prevalence of this phenomenon as well as its temporal dynamics are unknown. In this study, we used cell-to-cell comparisons of Endogenous Retrovirus (ERV) DNA methylation patterns in individual mice to show that silencing goes from stochastic to stable as insertions age. Variable methylation is frequent among recent insertions, which can show different methylation levels between mice as well as between cells of a same individual. Older insertions, in contrast, show stable methylation patterns both between cells and between individuals. These data are consistent with a proposed scenario in which variable methylation is due to a molecular conflict between forces promoting and opposing methylation. Our results emphasize the significance of previous studies demonstrating that TEs can contribute to phenotypic variability through their stochastic silencing. Indeed, variable methylation between individuals mediated by TEs is not a sporadic phenomenon but likely occurs for many recent TE copies. These findings also suggest that TE-mediated epigenetic variability is a dynamic phenomenon and affects different loci over time.

Unexpected Resolution of Neuroimaging Findings in a Patient with L-2 Hydroxy Glutaric Aciduria 12 Years Later. *A. Rajadhyaksha, E. Pacheco, K. Wierenga, P. Jayakar* Miami Children's Hospital, Miami, FL.

Introduction: L-2 Hydroxyglutaric Aciduria is rare neurodegenerative autosomal recessive disorder reported in less than 100 individuals. It is characterized by a static or slowly progressive course with psychomotor regression,mental retardation, seizures, macrocephaly and MRI findings of subcortical leukoencephalopathy with cavitations. Several studies have reported a correlation between the severity of clinical manifestations and the extension of the lesions by neuroimaging. **Case Report:** Patient RS first presented at 15(Hispanic & Scandinavian ancestry) with dysarthria, macrocephaly, seizures, intentional tremors and developmental delays. MRI showed extensive symmetrical signal abnormality involving the dentate nuclei of the cerebellar hemisphere, basal ganglia region, periventricular white matter and extension to cortical subcortical regions in the bifrontal regions, temporoparietal region and along the right insular cortex. Plasma amino acids showed increased lysine levels and urine organic acid revealed elevations of 2-OH Glutaric Acid confirmed by stable isotope dilution GC-MS revealing increases in L2 Hydroxyglutaric Acid enantiomer of 1623 mmol/mol creat (NL 1.3-19) and D2 Hydroxyglutaric Acid of 9.9 mmol/mol creat (NL 2.8-17). RS returned 12 years later for a reevaluation. Clinically he has done extremely well and has had no progression in symptoms. He is now 26 years old and drives to a job with a store. Though mildly delayed, he has graduated from school and completed some college courses. His seizures remain well controlled but tremors and mild dysarthria persists. A recent MRI revealed an improvement in the signal abnormality involving the dentate nuclei of the cerebellar hemisphere and basal ganglia but increased cerebral atrophy when compared to the prior scan. DNA mutation testing and repeat metabolic workup is in progress. **Conclusion:** With emerging treatment modalities, this disease should be part of a differential diagnosis in patients with macrocephaly and abnormal MRI. To our knowledge and a review of literature, there is no known case reported with improvements seen clinically and on neuroimaging studies.

Deep Re-Sequencing of Schizophrenia Candidate Genes. *J. J. Crowley[1,2], P. Sklar[3], S. Purcell[3,4], J. A. Lieberman[5], M. B. Morgan[6], O. Hall[6], L. R. Lewis[6], D. M. Muzny[6], A. Sabo[6], D. A. Wheeler[6], R. A. Gibbs[6], P. F. Sullivan[1]* 1) Genetics, University of NC, Chapel Hill, NC; 2) School of Pharmacy, University of NC, Chapel Hill, NC; 3) Psychiatric and Neurodevelopmental Genetics Unit, Massachusetts General Hospital, Boston, MA; 4) The Broad Institute of Harvard and MIT, Cambridge, MA; 5) Department of Psychiatry, Columbia University, and the New York State Psychiatric Institute, New York, NY; 6) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

There are ~10 genes for which there is compelling evidence for involvement in the etiology of schizophrenia (*COMT, DAOA, DISC1,DRD2, DRD3, DTNBP1, HTR2A, NRG1, SLC6A3, SLC6A4*). The pattern of results for these genes suggests that there may be sequence variants that have not yet been discovered, and existing re-sequencing have been limited. To address this limitation, as part of the NHGRIs Medical Re-Sequencing Project (in conjunction with the Gibbs lab at Baylor), we have re-sequenced the coding regions, 5 and 3 UTRs, splice sites, promoters and conserved intronic regions of these genes in 751 cases with schizophrenia from the CATIE trial and 747 controls. Traditional Sanger sequencing was used for SNP detection and 454 sequencing technology was used for SNP confirmation. In the schizophrenia cases, a total of 338 novel and 122 known SNPs were identified. The novel SNPs showed the following counts and average minor allele frequencies: intronic (176, 0.89%), coding non-synonymous (65, 0.35%), coding synonymous (52, 0.21%), splice site (15, 0.53%), 3UTR (14, 0.33 %), promoter region (13, 0.52%) and 5 UTR (3, 1.02%). Variants that were private to one individual comprised 190 (56%) of the novel SNPs. In total, cases possessed 21,491 minor alleles among these genes and 3,132 (15%) are from novel variants. Thus, each patient had about 2.5 known and 0.5 novel SNPs per gene. Analysis of the matched control data is in progress and will allow us to search for risk and protective factors for schizophrenia etiology. We aim to use a two-stage design where all significant and marginally significant findings from this cohort are followed up in larger case-control samples.

Homozygosity Mapping of Pigmented Hypertrichotic Dermatosis and Insulin-Dependent Diabetes Mellitus (PHID): Localization to a 1.4 Mb region of 10q22.1. *S. Cliffe[1, 2], T. Roscioli[1, 2, 3], J. Prendiville[4], M. Wong[5], K. Hussain[6], C. James[6], R. C. M. Henekam[6, 7, 8], M. F. Buckley[1, 2], R. Lindeman[2, 9]* 1) Molecular & Cytogenetics Unit, Department of Haematology and Genetics, South-Eastern Area Laboratory Service, Randwick, Sydney, Australia; 2) Centre for Vascular Research, University of New South Wales, Sydney, Australia; 3) Department of Molecular and Clinical Genetics, Royal Prince Alfred hospital, University of Sydney, Sydney, Australia; 4) Division of Pediatric Dermatology, Department of Pediatrics, British Columbias Childrens Hospital, and University of British Columbia, Vancouver, Canada; 5) Department of Immunology and Allergy, Childrens Hospital Westmead, Sydney, Australia; 6) Department of Endocrinology, Great Ormond Street Hospital for Children NHS Trust and the Institute of Child Health, University College London, London, UK; 7) Clinical and Molecular Genetics Unit, Institute of Child Health, London, UK; 8) Department of Paediatrics, Academic Medical Centre, Amsterdam, The Netherlands; 9) School of Medical Sciences, University of New South Wales, Sydney, Australia.

Pigmented hypertrichotic dermatosis and insulin-dependent diabetes mellitus (PHID) is an autosomal recessive condition that has been reported in 5 families. The dermatosis documented in PHID consists of pigmented hypertrichotic patches and induration on the upper inner thighs. Insulin-dependent diabetes mellitus (IDDM) is observed in 83% of cases with an age of onset of 9-15 years. Pancreatic hypoplasia has been reported in 2 of 6 patients with no evidence of pancreatic autoantibodies. Consanguinity is present in 4 families. High-density SNP genotyping was used to define regions of identity by descent in the affected individuals. This analysis identified a single shared 6.6Mb region at Chr10q22.1, encompassing 62 known and predicted genes. 12 of these genes were selected for sequencing on the basis of tissue expression and predicted function. No protein-coding sequence variations were detected, however heterozygous intronic SNPs further defined the critical region to a 1.4 Mb region containing 14 genes - sequencing of these genes is underway.

Acceptability of Breast Cancer Risk Assessment Using an Internet Family History Tool. *G. L. Wiesner[1], C. Simon[2], A. Lynn[3], L. Acheson[3]* 1) Dept. of Genetics; 2) Dept. of Bioethics; 3) Dept. of Family Medicine, Case Western Reserve University, Cleveland, OH.

Internet methods of family history risk collection can identify high risk women who would benefit from increased surveillance and genetic testing. This study assesses the acceptability and feasibility of using a computer-assisted family history tool to detect high risk patients in a busy breast clinic. A qualitative semi-structured interview was performed with 65 women (40% Af-Am; 48% college) about the potential use of an Internet tool to collect, store and analyze family history data for cancer risk. 12% felt their family history of cancer was absolutely/mostly private; 97% were comfortable providing family history of cancer to their own doctor and 97% felt that their relatives would also be very/mostly comfortable with them sharing the relatives cancer history with their doctor. 1595 consecutive women attending the clinic were then invited to input their family cancer history into the validated GREAT (Genetic Risk Easy Assessment Tool) that calculates breast cancer/BRCA mutation probabilities and generates a personalized report. 51% of the 1595 women had a family or personal history of cancer. 326 women responded to the invitation with 116 consenting and 194 declining (mean age 56, 43% Af-Am); 37% did not have a computer/internet access, and 26% were concerned about privacy/internet security. To date, 76 have completed the GREAT (mean age 56, 16% Af-Am, 22% with a history of breast cancer); 28% received reports indicating increased risk for HBOC; all were considered appropriate for genetic counseling. >90% found the GREAT easy to navigate, personal report easy to understand, and the time taken not lengthy. Most planned to talk with family members and show the report to relatives. Our quantitative study supports our qualitative analysis, in that most women found the web-based GREAT an acceptable means of personal and family cancer risk assessment in the breast clinic. Lack of internet access and concerns about security posed barriers for some would-be participants. Ongoing investigations are aimed at understanding the concerns of women at risk for breast cancer.

SNP genotyping in a proband with apparently balanced translocation identifies a microdeletion in 3q and implicates ALCAM in delayed cranial ossification. *N. Sobreira[1], E. Squibb[2], G. Steel[1], A. Hamosh[1], G. Thomas[2], D. Valle[1]* 1) Johns Hopkins University, Inst. of Genetic Medicine, Baltimore, MD; 2) Kennedy Krieger Institute, Baltimore, MD.

Apparently balanced de novo chromosomal translocations occur in approximately 1 in 2000 newborns and are associated with a 2-3 fold increased risk of malformations. We studied a 3 generation pedigree in which delayed cranial membranous ossification (OMIM 155980) cosegregates with an apparently balanced reciprocal translocation between 2p15 and 3q13.11. In addition to an abnormal skull the proband had thinning of the splenium of the corpus callosum, patent ductus arteriosus, and cataract. His sister, mother and maternal grandmother had a soft skull at birth and all had an apparently balanced translocation, t(2;3)(p15;q12). To access this rearrangement at higher resolution, we performed a whole genome SNP genotyping (Illumina 550) and found a region on 3q13.11 in the area of the cytogenetic breakpoint that showed 124 SNPs distributed over 550 kb with reduced intensity and apparently homozygosity. FISH confirmed the microdeletion predicted by SNP genotyping. This deletion at 3q13.11 removes ALCAM, a gene that encodes a cell adhesion molecule involved in osteoblast differentiation and CBLB, a gene that encodes an E3 ubiquitin-protein ligase. Using FISH probes in our patient, we narrowed the region of breakpoint at chromosome 2 to a region of 23 kb intergenic region. Of 9 previously reported patients with deletions involving the proximal segment of 3q13, no description of the shape or calcification of the skull was provided but 4 had agenesis of the corpus callosum. Although ALCAM and CBLB are both expressed in the brain, we speculate that ALCAM is more likely to be involved in this phenotype since it is also involved in osteoblast differentiation. In this case SNP genotyping, proved to be an excellent tool for precisely evaluating an apparently balanced translocation and revealed a cryptic deletion implicating candidate genes for this phenotype.

Lymphoblastoid cell lines as a proxy to brain for identification of genes in response to lithium treatment. *H. Chen[1], R. McEachin[1], N. Wang[2], X. Zhao[2], M. McInnis[1], M. Burmeister[1,2]* 1) Psychiatry, University of Michigan Medical School, Ann Arbor, MI; 2) Molecular and Behavioral Neuroscience Institute, University of Michigan Medical School, Ann Arbor, MI.

To date, study of lithium action has largely focused on animal models or postmortem human brain tissue. However, in future clinical practice, tissue from living patients will be required to assay genetic variation influencing treatment responses. Patients' brain tissue will not be available for such studies, so we explored the use of lymphoblastoid cell lines (LCLs) to identify genetic influences in lithium response. We assessed the effects of lithium on cell proliferation by culturing LCLs with the supplement of 0, 1, 2, 4, 6, and 10 mM LiCl, and microscopically counted the number of cells and estimated the portion of non-viable cells, respectively. In testing the hypothesis that lithium influences gene expression in this cell type, we assayed the expression of ~22,000 transcripts (genes) with the Illumina BeadChip microarray platform. We found that lithium treatment at 2 mM concentration was not grossly deleterious to cell proliferation, while at higher than 2 mM cell proliferation was inhibited. With the expression levels of ~22,000 transcripts (genes) determined, we found 36 genes significantly changed expression patterns in response to 1mM Li after applying a false discovery rate test (FDR < 0.05 and fold change > +/-15%). TaqMan assays confirmed 6 of 7 genes tested. Based on dbEST data, 33 of the 36 lithium-responsive genes in LCLs are also expressed in brain. Comparing our results to those seen in mouse brain, 11 of the 36 are common (same direction change) in both types of tissue. Functional analysis reveals transcription regulation as the main biological them discovered in this experiment. Our data supports the use of LCLs as a model for understanding the molecular basis of lithiums therapeutic action, and for identification of genetic variants to test allelic association with treatment responsiveness in bipolar disorder.

Assessing regulatory potential and functional consequences of Type 2 diabetes-associated variants on 9p21. *M. L. Stitzel[1], P. Deodhar[1], L. J. Scott[2], A. U. Jackson[2], N. Narisu[1], A. Swift[1], M. Boehnke[2], F. S. Collins[1]* 1) Genome Technology Branch, NHGRI/NIH, Bethesda, MD; 2) Dept. Biostatistics and Center for Statistical Genetics, University of Michigan, Ann Arbor, MI.

Variants within a 9.5 kb linkage disequilibrium block on chromosome 9p21, ~125 kb upstream of the heavily studied genes *CDKN2A/2B*, have been associated with type 2 diabetes (T2D) and decreased beta cell function in multiple studies and populations. Another gene on the opposite strand, *ANRIL*, is also a potential candidate for driving the T2D association, but its noncoding RNA is much less well characterized. Independent studies in mouse indicate that overexpression of *CDKN2A* (p16) inhibits beta cell proliferation. Taken together, these observations suggest the following hypothesis: (1) the associated region in humans harbors regulatory elements, (2) these elements regulate *CDKN2A*, and (3) the causal variant(s) increase expression in islets. We have cloned the entire T2D-associated LD block, as well as two smaller evolutionarily constrained sequences residing within this block, from risk and non-risk haplotypes present at ≥ 5% frequency in FUSION samples and tested them for enhancer activity in a minimal promoter luciferase assay in both beta cell-like (INS-1E) and non-beta cell (HeLa) lines. In preliminary experiments, we have observed a decrease in luciferase activity with these sequences. We are testing these elements for silencer activity and for specific enhancer/silencer activity of the *CDKN2A* promoter and cloning additional haplotypes present at a frequency of ≥ ~1% for testing. In addition, we are analyzing effects of the risk-associated variants on allele-specific and overall expression levels of *CDKN2A/B* and the putative noncoding transcript *ANRIL* in fibroblasts, peripheral blood lymphocytes, monocytes, adipose, and pancreatic islets. Though challenging, we expect these studies of allele-specific gene regulation in 9p21 will reveal possible mechanisms for T2D susceptibility.

Clinical Heterogeneity of Muenkes Syndrome in a Pregnant Mother and Her Neonate. *B. M. O'Brien[1], D. Abuelo[2], A. Oh[2], M. Vechiotti[2], C. Slack[1], N. Shur[2]* 1) Women and Infants Hospital at Brown,University, Providence , RI; 2) Hasbro Childrens Hospital at Brown University, Providence RI.

28 year old G3P1 presented in the mid-trimester for genetic counseling secondary to a history of craniosynostosis of an unknown suture. She underwent surgery at 12 weeks of age with no further surgeries or genetic evaluation. Her past medical history was also significant for Type I diabetes mellitus. She had no other abnormalities noted on exam, although she clearly had an abnormal head shape. Of note, she had normal appearing digits, normal hearing and had no learning difficulties. Her family history is significant for her 8 year old daughter (conceived with a different father) also having craniosynostosis, requiring two surgeries. A paternal uncle and his granddaughter also had craniosynostosis with a distant relative dyng in the neonatal period of "sudden death." The patient was counseled that it appeared that her family had an autosomal disorder, with a 50% risk that the fetus would inherit the disease causing gene. The patient declined any further investigation prenatally. She has a third trimester ultrasound that revealed subtle changes in the conformation of the fetal skull suggestive of craniosynostosis. In retrospect, it also appeared that there was midface hypoplasia. She was induced at 39 weeks due to maternal diabetes and the neonate had obvious dysmorphic features as well as craniosynostosis. His head circumference was at the 50th percentile, with brachycephaly and turricephaly. He displayed hypertelorism and significant hypoplasia of the midface. His neurological exam was nonfocal with no evidence of significant hypotonia. The diagnosis was confirmed with a Pro250Arg mutation in FGFR3. The child was admitted at one month of age with apnea episodes due to upper airway obstruction. He was found to have choanal stenosis and a deviated septum. Sudden death has been reported in association with upper airway obstruction in Muenkes. This case demonstrates the tremendous variability of Muenkes as well as the importance of counseling about the possibility of upper airway obstruction and even sudden death in this syndrome.

Inflammatory Gene Associations with Acute v. Chronic Coronary Artery Disease (CAD). *N. Ginwala[1], M. Li[1], M. S. Burnett[1], N. Mehta[1], A. Qasim[1], S. Restine[1], L. Pruscino[1], M. Wolfe[1], H. Chandrupatla[1], L. Satler[2], A. Pichard[2], R. Waksman[2], Z. Chen[1], J. He[1], J. Devaney[1], B. Keating[1], H. Hakonarson[1], S. E. Epstein[2], D. Rader[1], M. P. Reilly[1]* 1) Cardiovascular Medicine, University of Pennsylvania, Philadelphia, PA; 2) CVI/ MRI/Washington Hospital Center.

Background: Many inflammatory genes have been implicated in atherosclerotic CAD. It is unknown whether these genes relate to atherosclerosis or to acute clinical complications. Methods: The ITMAT Broad CARE (IBC) array is a 50K SNP array created to assess candidate genes (~2000) for cardiovascular disease. We examined inflammatory genes (N=27; adhesion molecules, chemokines, cytokines, metalloproteinases and their inhibitors, phospholipases, acute phase proteins) that may differentiate acute v. chronic CAD in a Caucasian, angiographic CAD case-control study (PennCATH: 509 acute cases, 497 chronic cases, 498 controls) with replication in a second CAD study (Medstar: 421 acute cases, 454 chronic cases, 447 controls) using Affymetrix 6.0 data. Results: There was no significant population stratification. In age and gender adjusted models in PennCATH, we found CAD associations for 19/27 genes, but with different patterns. Most genes related to both acute and chronic CAD [eg VCAM1 rs3917012[C] vs. control, all cases OR 1.31 (1.10-1.58 95% CI), p=0.003; acute cases 1.33 (1.07-1.65), p=0.01; chronic cases 1.30 (1.06-1.60), p=0.01]. Several genes showed differential associations, eg MMP3 was associated with acute [rs3020919[A], 1.36 (1.08-1.72), p=0.008], but not chronic CAD [0.97 (0.78-1.21), p=0.83; acute v. chronic p=0.006] whereas ICAM4 was associated with chronic [rs2228615[A], 0.84 (0.69-1.01), 0.05], but not acute CAD [1.02 (0.84-1.25), p=0.82; acute v. chronic p=0.008]. Of 19 genes associated with CAD in PennCATH, 11 replicated association in Medstar. Conclusion: We confirm associations of many inflammatory genes with CAD and show early evidence for differential associations with acute v. chronic CAD. The angiographic phenotype may aid in identifying genes for atherosclerosis versus those related to acute clinical complications only.

Induction of PGC-1 expression in Huntingtons disease transgenic mice rescues neuronal dysfunction and neurodegeneration. *T. Tsunemi[1], J. Au[1], A. R. La Spada[1,2]* 1) Department of Laboratory Medicine, University of Washington, Seattle, WA; 2) Center for Neurogenetics & Neurotherapeutics, University of Washington, Seattle, WA.

Huntingtons disease (HD) is an autosomal dominant disorder characterized by cognitive decline and involuntary movement. Expansion of a CAG repeat in the huntingtin (htt) gene is the cause of this disease. Pathogenesis results from production of htt protein with an expanded polyglutamine (polyQ) tract that misfolds and is resistant to proteasomal degradation. An important clue to selective neuronal vulnerability in HD has been mitochondrial oxidative phosphorylation defects in the striatum of HD patients, and modeling of HD in rodents by administration of mitochondrial toxins such as 3-nitropropionic acid. Another feature of HD pathogenesis is production of polyQ-expanded htt peptide fragments that localize to the nucleus and there disrupt transcription. Studies done on HD N171-82Q transgenic mice in our laboratory suggest that polyQ-expanded htt interferes with nuclear transcription programs responsible for mitochondrial biogenesis and oxidative phosphorylation. PPAR co-activator 1 (PGC-1) is a key transcription regulator of mitochondrial physiology, and appears to be the target of transcription interference by mutant htt protein. To test if restoration of PGC-1 function is sufficient to ameliorate neurological disease progression and striatal degeneration in HD mice, we have crossed HD N171-82Q mice with inducible PGC-1 transgenic mice. By incorporating Rosa26-rtTA mice into this breeding scheme, we derived triple transgenic mice whose expression of PGC-1 was found to be two times higher than N171-82Q controls upon doxycycline treatment. Analysis of littermate cohorts derived from these crosses shows that induction of PGC-1 expression significantly improves neurological function in HD transgenic mice, based upon rotarod performance and other motor tests. Surprisingly, PGC-1 over-expression also markedly decreased accumulation of misfolded htt protein in the brains of HD transgenic mice. Enhancing PGC-1 function could thus be a viable therapeutic intervention in HD patients.

Congenital Erythropoietic Porphyria: Viable Murine C73R Homozygotes, Knock-In Model of the Most Common Human Mutation. *D. Bishop, S. Clavero, R. J. Desnick* Genetics & Genomic Sciences, Mount Sinai School of Medicine, New York, NY.

Congenital erythropoietic porphyria (CEP) is an autosomal recessive disorder caused by the deficient activity of the fourth enzyme of heme biosynthesis, uroporphyrinogen III synthase (UROS). In humans, phenotypic severity correlates with the amount of residual UROS activity and varies from fetal lethality to only cutaneous lesions due to photodynamic damage upon exposure to sunlight. Many patients have transfusion-dependent disease. Bone marrow transplantation is curative but associated with increased morbidity and mortality. To develop animal models for therapeutic efforts, we used homologous recombination to create new models of murine CEP with varying severity and viabilities, including the homozygous viable C73R mouse that is the counterpart of the most common human CEP genotype. In addition, we have generated a homozygous viable V99L mouse and a homozygous lethal null mouse. The various combinations of these and previously reported hypomorphic alleles resulted in a range of phenotypes and demonstrated that less than 1% housekeeping enzyme is fetal lethal, even in the presence of 30% erythroid activity. The C73R/C73R mouse was obtained by interbreeding C73R/V99L mice on a mixed C57Bl/6, SV129, CD1, and FVB/N background. This mouse has a fetal viability of about 20% of wild-type, but once surviving past 5-10 days, appears to have a normal lifespan, albeit with reduced growth weights. The newborn mice are runted, pale, and anemic with an erythrocyte (RBC) count of 5.10.5x10^6/l that was half that of normal mice (9.80.9). The RDW was increased (36.91.3 vs 17.70.9%) due to microcytosis. The reduced RBC count and a 10x elevated platelet count (234 vs 19x10^3/l) suggested hemolytic anemia. The newborn C73R mice were highly fluorescent over their entire bodies and produced dark brown urine that contained high concentrations of uroporphyrins. Urinary URO I was 26718 nmoles/mg creatinine, but undetectable in normal mice. The URO I/III ratio was 7.81.0. COPRO I was 22.44.2 vs 0.30.1, while the COPRO I/III ratio in normal mice was 0.090.01. Due to the ease of genotyping by porphyrin fluorescence, they make attractive models for therapeutic endeavors.

A High-density Haplotype Resource of 94 Inbred Mouse Strains. *E. Kostem[1], H. M. Kang[1], A. Kirby[2], C. Wade[2], B. Han[1], M. Bogue[4], F. Johnson[7], K. Frazer[6], E. Beilharz[5], D. Cox[5], E. Eskin[1], M. Daly[2,3]* 1) Computer Science Department, University of California, Los Angeles, Los Angeles, CA; 2) Center for Human Genetic Research, Massachusetts General Hospital, Boston, MA; 3) Broad Institute of Harvard and MIT, Cambridge, MA; 4) The Jackson Laboratory, Bar Harbor, ME; 5) Perlegen Sciences, Inc., Mountain View, CA; 6) Scripps Genomic Medicine, La Jolla, CA; 7) National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina.

Due to the importance of the mouse as a human disease model, phenotypic variation among inbred mouse strains form a bedrock of modern medical research. A key step in determining the genetic underpinnings of this phenotypic variation is developing a catalogue of the genetic variation among inbred mouse strains. A recent whole genome resequencing study of 15 inbred mouse strains captured a significant fraction of the genetic variation among a limited number of strains. The availability of hundreds of inbred strains, including over 190 stocks available from the Jackson Laboratory, motivates the need of a high-density variation map for a larger set of strains. In this study, we collected a dense set of genotypes from 94 inbred mouse strains containing 10.77 million genotypes over 121,433 single nucleotide polymorphisms (SNPs), dispersed at 20kb intervals on average across the genome, with an average concordancy of 99.942% with previous SNP sets. Using an analysis of shared segments, we identified an average of 8.34 distinct segments over 94 strains, and 4.70 over 73 classical inbred strains across the genome, suggesting a limited genetic diversity between the strains. Combining with additional 7,570 gap-filling SNPs, we further imputed the untyped or missing genotypes of 94 strains over 8.27 million Perlegen SNPs. The average imputation accuracy among classical inbred strains is estimated to 97.8% overall. Approximately 70% of the genotypes (446,110,962 in total) are imputed with high confidence and achieve an accuracy of 99.7%.

***MYC* negative Burkitts lymphoma with 11q duplication involving the *MLL* gene.** *G. D'Amours[1,3], R. Absi[1], D. Dal Soglio[1], J.-C. Fournet[1], M. Blagdon[2], E. Lemyre[3], R. Fetni[1]* 1) Département de Pathologie; 2) Département d'Hématologie; 3) Service de Génétique médicale, CHU Sainte-Justine, Université de Montréal, Québec, Canada.

Burkitts and Burkitt-like lymphoma represents approximately 40% of non-Hodgkins lymphomas (NHL), and 3-4% of all childhood malignancies. Its diagnosis is based on clinical presentation, morphological and immunophenotypic features, combined with cytogenetic and molecular findings. Most cases involve translocation of the *MYC* gene, located at 8q24, to an immunoglobulin locus. Classic translocation involves 14q32, and is present in 85% of *MYC* positive cases. Less frequently, the translocation partner is either chromosome 2 at band q11, or 22q11, in 5% and 10% of cases respectively. Only 10% of Burkitts lymphomas are negative for this rearrangement. Little data are available on the oncogenic mechanism of the *MYC* negative Burkitts lymphomas. We report the case of a 12-year-old boy, who presented with pain in the right thigh, limp, fatigue and weight loss. Clinical exam revealed a mass on the right iliac fossa and splenomegaly. There was no biopsy of the principal mass performed. Bone marrow morphology and immunophenotype were suggestive of Burkitts lymphoma, except for the absence of IgM. FISH analysis showed the absence of *MYC* rearrangement. G-banding karyotype revealed an abnormal chromosome 11, consisting of either a derivative from a t(11;12) or an 11q duplication. Analysis with whole chromosome 12 painting excluded the possibility of a t(11;12). In order to characterize the segment duplicated, the LSI *MLL* (11q23) Dual-Color Break Apart Probe was used. FISH analysis showed 3 *MLL* signals in 77% of interphase nuclei, with two of these signals in close proximity. Analysis of metaphase cells and the order of the green and red signals on interphase nuclei revealed an inverted duplication of 11q13q23.3, resulting in trisomy of this segment, with a telomeric breakpoint distal to *MLL* locus. Trisomy 11q is a rare but recurrent imbalance in B cell tumors. Further cytogenetic and molecular investigations of negative *MYC* Burkitts lymphomas could help to characterize this entity of B cell tumors.

Interaction between IRF6 and TGFA contributes to the risk of cleft lip/palate. *A. Letra[1], R. F. Fonseca[3], R. Menezes[1], T. H. McHenry[1], S. Daack-Hirsch[4], M. L. Marazita[1,5], E. E. Castilla[6], B. C. Schutte[7], I. M. Orioli[3], J. M. Granjeiro[8], A. R. Vieira[1,2,7]* 1) Dept Oral Biology and Center for Craniofacial and Dental Genetics, Univ of Pittsburgh School of Dental Medicine, Pittsburgh, PA; 2) Dept Pediatric Dentistry, Univ of Pittsburgh School of Dental Medicine, Pittsburgh, PA; 3) ECLAMC at Dept Genetics, Federal University of Rio de Janeiro, RJ, Brazil; 4) College of Nursing, Univ Iowa, Iowa City, IA; 5) Dept Human Genetics, Graduate School of Public Health, Univ Pittsburgh, Pittsburgh, PA; 6) ECLAMC at Oswaldo Cruz Foundation, RJ, Brazil; 7) Dept Pediatrics, College of Medicine, Univ Iowa, Iowa City, IA; 8) Dept Cell and Molecular Biology, Fluminense Federal University, RJ, Brazil.

Studies of tooth agenesis suggest that IRF6 and TGFA may interact. Tooth agenesis is commonly found in individuals with CL/P; hence we used three cohorts to evaluate if IRF6 and TGFA interaction also contributes to CL/P. Markers in/nearby IRF6 and TGFA were tested for case-control analyses in 1,000 Brazilian individuals. We looked for evidence of gene-gene interaction between IRF6 and TGFA in our cohort and additionally in 142 case-parent triads from South America and 861 families by testing if associated markers were overtransmitted together. Irf6 and Tgfa expression was analyzed by immunohistochemistry in normal and Irf6 knockout mice. Both IRF6 and TGFA showed association with CL/P subtypes in the Brazilian cohort (p=0.00001). Statistical evidence of interaction was found for all data sets (p=0.01 for Brazil; p=0.046 for South America, and p=0.003 for the 861 families). Attributable fraction calculations suggest that such interaction may explain 1% - 10% of all CL/P cases. Expression of Tgfa was decreased in embryos that lacked Irf6. We provide further evidence that IRF6 and TGFA contribute to CL/P. Moreover, IRF6-TGFA interaction appears to contribute to as much as 1% to 10% of CL/P cases. The IRF6-knockout model further support the evidence of IRF6-TGFA interaction we found in humans. NIH grants R21-DE016718, R01-DE016148, P50-DE016215; CAPES/BEX3413055, CNPq 30885/2006-6, FAPERJ (E-26/152.831/2006).

A robust copy number variation discovery algorithm for multiple array platforms. *D. Pinto, J. Zhang, B. Thiruv, Z. Wang, L. Feuk, P. Hu, C. Greenwood, S. W. Scherer* The Centre for Applied Genomics and Program in Genetics and Genome Biology, the Hospital for Sick Children and University of Toronto, ON, Canada.

Developments in microarray design and data analysis are crucial for accurately detecting copy number variation (CNVs) on a genome-wide scale. There is an unmet need for algorithms that can detect CNVs with high sensitivity and specificity. We have developed an algorithm, *iPattern*, which calls CNVs from intensity data derived from SNP or CGH arrays. *iPattern* uses non-parametric density-based clustering to categorize sample loci into different groups when compared to reference samples by using an optimal moving window-based approach. The largest cluster of unrelated samples is chosen as reference, and samples with higher or lower intensities are assigned as relative CNV gains or losses. *iPattern*'s performance was evaluated using simulated and real data. Simulation studies were carried out on synthetic data derived from various X chromosome copies, and clustering parameters were chosen to maximize sensitivity while setting the false discovery rate (FDR) to 3%. *iPattern* was tested on HapMap data generated using the Affymetrix SNP 6.0 (268 samples) and the Illumina Beadchip 1M (103 samples). From these datasets we detected 28,331 and 4,719 CNVs, respectively, accounting for 106 and 46 CNVs/sample. About 90% of the calls from both platforms overlapped known CNVs in the Database of Genomic Variants. The quality of CNV calls was further assessed by examining inheritance patterns in parent-child trios from control data and in a disease cohort of 350 families. The fraction of CNV calls in children not detected in parents was 8.3%, a significant improvement over other available tools. Considering that an unknown proportion of these calls consist of *de novo* CNVs, the actual sum of false discoveries (i.e. combination of false-positive and false-negative rates) is likely lower. Finally, sensitivity and specificity estimates using replicate samples indicated that *iPattern* had 1.7 times higher sensitivity than that of another method at a similar FDR. Our data show that *iPattern* is currently the most robust CNV calling algorithm for analysis of Affymetrix SNP 6.0 and Illumina 1M array data.

Genomic imbalance as a cause of fetal and infant death. *J. Gerfen[1], L. K. Conlin[1,2], M. Berman[2], D. DiPatri[1], S. Kasperski[1], R. D. Wilson[1], L. Ernst[1], N. B. Spinner[1,2]* 1) The Childrens Hospital of Philadelphia, Philadelphia, PA; 2) University of Pennsylvania School of Medicine, Philadelphia, PA.

The cause of fetal and neonatal death is unknown in 50% of cases. While cytogenetic abnormalities make a significant contribution to early fetal loss (peak frequency at 12-15 weeks), chromosome abnormalities play a lesser role in fetal loss after 24 weeks. Recent technologic advances offer the opportunity to search for smaller genomic imbalances that would have been missed by chromosome analysis. We used genome-wide SNP array analysis to study a cohort of 111 patients who died before birth, within the first year of life, or following pregnancy termination due to multiple malformations. We identified an average of five copy number alterations (10 SNPs for heterozygous calls, 5 SNPs for homozygous deletions) per patient. To prioritize the potential significance of the deletions and duplications identified (pathogenic versus benign copy number variation), we developed a program called PErl COpy Number of Potential Interest (PECONPI). PECONPI assigns a score to each deletion or duplication based on its frequency in controls (in house and literature), genetic content, size, and number of copies. Higher PECONPI scores indicate a higher likelihood to be pathogenic. We found 549 genomic deletions or duplications, and 56 were scored to indicate exonic involvement with no overlap with controls. So far, we have identified six abnormalities that we believe were the cause of death, including a homozygous deletion of the glycine decarboxylase gene in a neonate who died of non-ketotic hyperglycemia, a mosaic deletion of 11q in a patient who died of neuroblastoma, and a 2q14 deletion including the Gli2 gene in a stillborn fetus with a brain malformation. Parental samples were available for five patients, two of whom had duplications that were potentially pathogenic based on literature reports (3q29, 16p11.2). Both variants were inherited from a phenotypically normal parent and thus it is not likely to be the cause of demise for these individuals. Work is ongoing to investigate the remaining patients and to further characterize the role of genomic imbalances in fetal and infant death.

Glutaric Aciduria Type II presenting with lipid myopathy, progressive leukodystrophy; intrafamilial variation in two siblings. *W. Al-Hertani[1], A. Mineyko[2], P. Humphreys[2], P. Chakraborty[1], M. T. Geraghty[1]* 1) Dept Medical Genetics, CHEO, Ottawa, ON, Canada; 2) Department of Neurology, Childrens Hospital of Eastern Ontario, Canada.

Glutaric Aciduria Type II (GAII) is an autosomal recessive disorder caused by a deficiency of Electron Transfer Flavoprotein (ETF) or ETF-ubiquinone oxidoreductase (ETFQO). ETF and ETFQO play an important role in the transfer of electrons from the breakdown of fats and proteins to ubiquinone in the respiratory chain. We report a 4 year old female who presented at 6 months with significant hepatomegaly, hypotonia, profound myopathy requiring ventilation for 2 months. At presentation there was a mild hyperchloremic acidosis with elevated muscle enzymes. Plasma glucose, ammonium, lactate and amino acids were normal. Urine organic acids showed dicarboxylic aciduria. Plasma acylcarnitines showed elevations in long chain species consistent with atypical GAII or a long chain defect. EMG showed no activity while nerve conduction was normal. Liver biopsy showed moderate to severe fatty infiltration. Six weeks into the course muscle biopsy showed a resolving lipid myopathy with mild mitochondrial changes on electron microscopy. Deficiencies in Complexes I/III and II/III were reported in muscle but not liver. Several months after recovery brain MRI showed a progressive leukodystrophy. CoQ levels at that time were normal in muscle and leukocytes. Analysis of fatty acid oxidation in fibroblasts was reported as normal in 2 laboratories but abnormal and non diagnostic in a third. Western blot and enzyme activity levels showed normal ETF but mildly reduced ETFQO levels and low activity (0.44nmol/min/mg; normal 0.8-2.4; typical affected:<0.2). Sequence analysis of the ETFDH gene showed 2 mutations. One is a previously reported mutation (nt51insT) the other (E314K) has not been published to date. The probands 9 year old brother has both mutations, abnormal acylcarnitines and absent ketones on overnight fasting, but is asymptomatic. Because of advancing leukodystrophy unresponsive to high dose riboflavin and CoQ, the proband was started on D,L betahydroxybutyrate and we are following the response.

Comparison of BiGS GWAS results with other GWAS studies of Bipolar Disorder. *D. L. Koller[1], T. Foroud[1], X. Xuei[1], H. J. Edenberg[1], J. I. Nurnberger[1], W. Berrettini[2], W. Byerley[2], W. Coryell[2], E. Gershon[2], W. Lawson[2], M. McInnis[2], F. McMahon[2], J. P. Rice[2], W. Scheftner[2], P. Zandi[2], N. Schork[3], J. Kelsoe[3]* 1) Indiana Univ Sch Medicine, Indianapolis, IN; 2) BiGS Consortium; 3) Univ Calif-San Diego.

We have recently completed genotyping of a case-control study of bipolar disorder (the BiGS study, dbGAP filtered dataset,n=1021 cases); primary results are presented in a companion abstract. GWAS studies of bipolar disorder have been published by the Wellcome Trust Case Control Consortium (Nature 447:661, n=1868) and the StepBD consortium (Mol Psych 13:558-569, n=1461). We compare results from these three studies, all of which focused on type I bipolar cases. Loci with evidence of association across multiple bipolar GWAS were identified by computing the product of the three allelic association P-values for SNPs included in the Affymetrix 500K array (used for WTCCC and StepBD), which are a subset of the Affymetrix 6.0 array (BiGS). Chromosomal regions were identified containing multiple SNPs with P-value products less than 10^{-6}, to prevent the detection of single SNPs with little or no support from nearby markers. We then further restricted our interest to regions achieving a nominal p-value less than 0.01 in the BiGS study and with the same risk allele detected in at least one of the published studies (Wellcome or StepBD). We detected 8 regions meeting these criteria in BiGS and one of the other two studies, and 2 regions (*SYNE1* and *CDH11*) with association evidence from all three studies. Notably, evidence of replication across the bipolar GWAS studies occurs primarily for SNPs with p-values that do not reach the thresholds for genome-wide significance. The vast majority of the findings were not among the top 1000 SNPs in the respective studies. This is consistent with the conclusions of Baum et al. (Mol Psych 13:466). We conclude that follow-up studies should include these commonly detected regions, as well as candidates with evidence of association from non-GWAS studies, and not focus entirely on the chromosomal regions providing the highest level of statistical significance in any one study.

Analysis of the EAAT2 glutamate transporter (*SLC1A2*) locus as an 11p13 positional candidate for autism. *G. D. Schellenberg for the Autism Genome Project* U Pennsylvania.

Autism is a developmental disorder defined by deficits in reciprocal social behaviors, communication and patterns of repetitive behaviors and restricted interests. Approximately 1 in 150 individuals are affected by an autism spectrum disorder (ASD), with twin and family studies indicating a predominantly genetic etiology. The Autism Genome Project (AGP), a large international collaboration, previously reported a Phase I linkage analysis on a sample of >1100 multiplex families (*Nat Genet* 39: 319, 2007). In addition to ancestry and gender effects on linkage, the most prominent specific locus to emerge corresponds to a region on 11p13. Peak linkage (Z_{lr} = 3.57) was observed at marker rs2421826 at 52 cM, with a significant increase observed in a subset of families with one or more affected females (Z_{lr} = 4.37). Of the genes located in the region flanking peak linkage, *SLC1A2*, which encodes the EAAT2 glutamate transporter, emerged as a plausible candidate gene. To test the hypothesis that *SLC1A2* harbors common and/or rare alleles conferring risk for autism, the AGP performed allelic association and exploratory resequencing analyses. Resequencing studies involved a sample of 200 unrelated probands and 100 unrelated control subjects and amplimers covering all coding and 5 untranslated exons. Several novel synonymous and nonsynonymous variants were detected in probands, however, the observed variation does not suggest a likely mutation burden. Association analysis was conducted using 35 SNPs chosen to index common haplotypes across ~85 kb of exonic and 5 and 3 flanking sequence. Genotype data from a combined multiplex/simplex sample of 2,668 families was assessed for quality control and Hardy-Weinberg. Resulting data were analyzed using FBAT based on stringency of autism diagnoses and revealed an excess of nominally-significant P-values across a large block of linkage disequilibrium, and in particular its 5 end. Ten SNPs demonstrated P-values <0.05 and five SNPs with P-values <0.01. These results, and substantial linkage to autism at 11p13, provide compelling but not fully convincing evidence that variation in *SLC1A2* affects autism susceptibility.

A 5.7Mb deletion at chromosome 14q22-q23 in a child with short stature, polydactyly, and mild dysmorphic features. *A. N. Filose, G. E. Tiller* Department of Genetics, Kaiser Permanente, Los Angeles, CA.

We describe a 4 year old boy with a 5.76Mb deletion on chromosome 14 using comparative genomic hybridization (CGH). Clinical evaluation as an infant revealed hypotonia, large anterior fontanelle, hypertelorism, distal postaxial polydactyly, and normal brain MRI and 46,XY karyotype. Subsequent evaluation at 3 years of age was remarkable for short stature, mild developmental delay, and dysmorphic features including relative macrocephaly, mild proptosis, hypertelorism, prominent forehead, and long narrow nose with anteverted nares. A formal ophthalmologic examination was unremarkable. CGH analysis revealed a de novo 5.76Mb interstitial deletion extending from 14q22.1 to 14q23.1. Approximately 35 genes are within the deleted region, including SPG3A, PYGL, BMP4, and GCH1. Most notable of these is BMP4, in which mutations are associated with eye, brain, and digital anomalies. Deletions in this region are rare, with perhaps 11 cases described in the literature to date. The 14q deletions previously reported are similar in size and location to this patient. The majority of cases described exhibit bilateral anophthalmia; unilateral anophthalmia, brain anomalies, developmental delay, and polydactyly are also frequent, but not universal findings. This case may represent a milder form of an emerging deletion syndrome.

The effects of linkage disequilibrium in large scale SNP datasets for Multifactor Dimensionality Reduction. *B. J. Grady, E. S. Torstenson, M. D. Ritchie* Center for Human Genetics Research, Vanderbilt University, Nashville, TN.

In the analysis of genome-wide association studies (GWAS), an important consideration is the power of analytical methods to identify correct predictive models of disease. When trying to get power from such analytical methods, a confounding factor up to this point has been the presence of linkage disequilibrium in GWAS datasets. In order to look at the effect of LD on association analysis, in particular with respect to detecting gene-gene interactions, the genomeSIMLA data simulation software package was used to simulate SNPs in LD and data was then analyzed for two-locus interactions using Multifactor Dimensionality Reduction (MDR). MDR is a non-parametric statistical method for detecting gene-gene and gene-environment interactions. MDR performs a dimensional reduction by assigning multi-locus genotypes to either high or low risk groups and using this model to predict disease status. Using genomeSIMLA, we simulated datasets with varying proportions of SNPs in LD in which between 15% and 95% of the SNPs were in LD with at least one other SNP in the dataset, where LD is defined by an r2 of at least 0.8. In addition, we simulated three different scenarios for the disease susceptibility loci: a model in which both SNPs are in separate blocks of LD; a model with one SNP in an LD block and the other not in LD with any other SNPs; a model of two SNPs in the same block of LD. Multiple penetrance functions were then used to create varying effect sizes. Results from these analyses indicate that higher levels of LD begin to challenge the MDR algorithm such that the ability to detect the functional locus is decreased; however, there is ample power to detect the SNPs in the blocks of LD with the functional locus. This simulation study indicates that the use of MDR in large scale SNP data with varying amounts of LD can be fruitful as long as one pays attention to the LD patterns within the dataset.

Analysis of Copy Number Variants (CNVs) in a Genomewide Association Study of Bipolar Disorder (BPD). *K. P. Shah[1], D. Absher[5], J. Xu[1], M. Bennet[6], L. J. Scott[2], R. C. Thompson[3], W. Guan[2], M. Flickinger[2], F. Meng[4], A. Southwick[6], A. F. Schatzberg[7], J. D. Barchas[8], W. E. Bunney Jr.[9], E. G. Jones[10], M. Burmeister[3,4], H. Akil[4], S. J. Watson[4], M. Boehnke[2], R. M. Myers[6], J. Li[1]* 1) Dept of Human Genetics; 2) Biostatistics; 3) Psychiatry; 4) Molecular & Behavioral Neuroscience Inst, Univ. of Michigan, Ann Arbor, MI; 5) HudsonAlpha Inst. for Biotechnology, Huntsville, AL; 6) Stanford Human Genome Ctr; 7) Dept of Psychiatry and Behavioral Sciences, Stanford Univ., Palo Alto, CA; 8) Weill Medical College, Cornell Univ., New York, NY; 9) Dept of Psychiatry & Human Behavior, Univ. of California, Irvine, CA; 10) Ctr. for Neuroscience, Univ. of California, Davis, CA, USA.

We performed a GWAS of BPD involving genotyping 550,000 SNPs in 1,147 BPD cases and 778 unrelated controls. Samples are European Americans frequency-matched by self-reported ancestry. SNP-based analysis results are reported in a separate abstract (Scott et.al.). As CNVs may account for additional disease risks we set out to examine their potential contribution to BPD. From genotyping intensity data we identified 41,412 CNVs, averaging 22 per sample. Of these, 29,541 common CNVs, defined as spanning 2 SNPs and occurring in 3 samples, fall into 1,700 unique loci. The remaining 11,871 CNVs, seen in only 1-2 samples, fall into 10,436 rare CNV loci. Together the 12,136 CNV loci cover 623 Mb (22% of the genome). 54% have not been reported before (Database of Genomic Variants); 43% are within 5 kb of RefSeq genes. About 78% contain deletions, 22% contain amplifications, and 1% contain both. A higher proportion of rare CNV loci are near genes (45% vs 32%) than common CNV loci, suggesting differences in selective pressure in genic regions. Preliminary case-control comparisons of CNV frequencies revealed none that reached genomewide significance. To increase power, we are currently combining our study with other GWAS of BPD to detect signals of association for both SNPs and CNVs. The authors are members of the Pritzker Neuropsychiatric Disorders Research Consortium, which is supported by the Pritzker Neuropsychiatric Disorders Research Fund L.L.C.

Mosaic Chromosome 5q Deletion in Mother and Daughter Delineated by Genomewide SNP Microarrays. *R. Lebo[1], J. Collins[2], M. Khalifa[3], P. Hong[1], P. Cifuentes[4], E. Mansfield[5]* 1) Dept Pathology, Akron Children's, Akron, OH; 2) Div Molecular Diagnostics, Affymetrix, Santa Clara, CA; 3) Div Genetics, Dept Pediatrics, Akron Children's; 4) Div Cytogenetics, Affymetrix; 5) Div Scientific Applications, Affymetrix.

Mosaic chromosome 5q microdeletion in mother and daughter was identified by a 437 BAC site Spectral Genomics microarray. The mother and daughter were confirmed to share mosaic microdeletions by BAC FISH to metaphase cells. The daughter has bilateral clinodactyly of the little finger, partial clinodactyly of the second and third toes, downward slanting palpebral fissures, cup shaped prominent ears, micrognathia with elongated smooth filtrum and prominent maxilla, and delayed speech development. Both parents are phenotypically normal. To delineate these deletions, confirm paternity, and identify de novo mutations, the Affymetrix 6,000,000 site SNP 6.0 microarray was employed. Mosaic deletion of the 125 kb region in chromosome band 5q23.3 disrupting the ADAMTS19 gene was again confirmed in both the mother and daughter. ADAMTS19 is a metalloproteinase involved in tissue remodeling and angiogenesis and expressed most abundantly in fetal tissue. Several additional de novo duplication and deletion sites were found in the daughter as well as multiple copy-neutral LOH regions including three Megabase regions on Xp11.13, Xq22.3 and 3p21.1. These gene rich regions encode multiple cell cycle regulators (PARP3, PBRM1, RASSF1,TUSC4), DNA repair genes (BAP1, PBRM1, PCBP4, RBM6) and several candidate mental retardation genes involved with axon guidance (SEMA3F, RHOA,SEMA3B, GNAI2, SEMA3G) or tight junction formation (EPB41L1, RHOA, CASK, GNAI2, MYL9). Together the concert of molecular changes in the daughter's genome identified with the Affymetrix SNP array could explain her abnormal phenotype. This case demonstrates (1) the application of genomewide SNP analysis to obtain data quickly and thoroughly on complex cases, (2) confirming mosaic deletions by FISH analysis of metaphase chromosomes, and (3) an abnormal phenotype may require extensive analysis to determine the source.

Multiple, rare, segregating variants may be responsible for familial combined hyperlipidemia. *E. Wijsman[1], J. Rothstein[1], J. Brunzell[1], R. Krauss[2], A. Lusis[3], A. Motulsky[1], G. Jarvik[1]* 1) Univ. of Washington, Seattle, WA; 2) Univ. of California, Berkeley, CA; 3) Univ. of California, Los Angeles, CA.

Cardiovascular disease remains the leading cause of death in Western societies. Familial combined hyperlipidemia (FCHL) is a complex disorder leading to increased cardiovascular disease risk. It is characterized by the presence of elevated cholesterol, triglyceride (TG), and apolipoprotein B (APOB) levels, with also associated changes in low-density lipoprotein (LDL) levels and size, measured as peak particle diameter (PPD). We previously showed evidence (lod > 3 or log Bayes factor, BF > 1) for linkage of PPD to chromosome (chr) 9 in a single pedigree, and to chr 11 jointly in 4 large pedigrees (N=255 individuals), as well as suggestive evidence for linkage to chr 14 and 16 [ATVB 24:1942, 2004]. Here we extend analysis to additional phenotypes (logTG, APOB), to additional markers, and to 18 additional pedigrees (N=542). We used whole chromosome multipoint Markov chain Monte Carlo (MCMC) methods to compute parametric lod scores and BFs for joint oligogenic segregation and linkage analysis (OSLA). APOB was adjusted for LDL and PPD, PPD was adjusted for logTG, and all 3 traits were adjusted for age, sex, and BMI prior to analysis. Additional dense genotyping increased evidence for linkage of PPD to chr 9 (lod=4.07) and narrowed the location to 28-37 cM, and analysis of all 22 pedigrees identified chr 11 at ~115 cM as the region with strongest linkage evidence for PPD and log TG across all pedigrees (log BF=2.25 and 1.5, respectively). Additional evidence of linkage of logTG across all families was also obtained for chr 7 (log BF=1.5) and 14 (log BF=1.5), and strong support for linkage in one or a few families was obtained for PPD (chr 10p, log BF=2.5) and for APOB (log BF=1.5, lod=3.1). Each large pedigree typically supported linkage to at least two genomic locations with log BF=1-2 and with identifiable models from OSLA, but with little overlap in the identified regions across families. These results suggest that multiple rare variants are segregating, and that use of large pedigrees may be the most useful strategy to identify causal variants for these traits.

Identification of *VANGL1* variants associated with neural tube defects. *Z. Kibar[1], M. Kooistra[2], P. Fortin[2], S. Salem[2], P. De Marco[3], E. Merello[3], A. G. Bassuk[4], V. Capra[3], P. Gros[2]* 1) Obstetrics and Gynecology, CHU Sainte Justine and University of Montreal, Montreal, QC, Canada; 2) Department of Biochemistry, McGill University, Montreal, QC, Canada; 3) Laboratorio del Servizio di Neurochirurgia, Instituto G. Gaslini, Genova, Italy; 4) Department of Pediatrics, University of Iowa, Iowa City, Iowa, USA.

Neural tube defects (NTDs), including spina bifida and anencephaly, represent a group of common and severe congenital malformations affecting 1-2 per 1000 birth. They are caused by failure of the neural tube to close during early development. Their etiology is complex involving both environmental and genetic factors. We have recently reported three mutations in the planar cell polarity gene *VANGL1* in NTDs, which are most likely pathogenic based on genetic and functional data. To better understand the role of *VANGL1* in the development of NTDs, we analyzed this gene by direct sequencing in a large and well-characterized cohort affected with various forms of NTDs including cranial, open and closed spinal dysraphisms. The cohort consisted of 219 Italian patients recruited at the Spina Bifida Center of the Gaslini Hopsital in Genova, Italy and 480 patients recruited at the Children's Memorial Hospital in Chicago, Illinois, United States. The latter included patients of various ethnic origins including Caucasian, African Americans and Latin Americans. The control group consisted of 205 unrelated healthy random Italian individuals and 1200 individuals from the Human Genome Diversity Project. Sequence analysis of *VANGL1* identified four variants, R181Q, L202F, T251M and R517C,that were absent in all ethnically-matched controls analyzed. These variants affect highly conserved amino acid residues. We are currently validating the pathogenicity of these variants by various biochemical assays in vitro and in vivo. We are also conducting a dense single nucleotide polymorphism (SNP) association study (10 SNPs) to identify alleles, genotypes and haplotypes at *VANGL1* that confer a high risk for NTDs. Our preliminary results provide further evidence supporting the role of *VANGL1* as a strong player in development of spinal NTDs.

Linkage and Association on Chr 8p21 in Schizophrenia. *M. D. Fallin[1,4], V. K. Lasseter[3], D. Avramopoulos[2,3], P.-L. Chen[2], K.-Y. Liang[4], Y. Liu[2], J. McGrath[3], G. Nestadt[3], P. Wolyniec[3], D. Valle[2], A. E. Pulver[3]* 1) Dept of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; 2) Institute of Genetic Medicine, Johns Hopkins School of Medicine, Baltimore, MD; 3) Dept of Psychiatry, Johns Hopkins School of Medicine, Baltimore, MD; 4) Dept of Biostatistics, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD.

Many linkage studies have identified a locus for schizophrenia (SZ) on Chromosome 8p21, including a recent ~6000-SNP linkage analysis of 831 pedigrees from 8 site that showed linkage to a 1Mb region from rs1561817 to rs9797 (Zmax=3.2, p=0.0004). Our 132 European Caucasian families from Johns Hopkins (JHU) contributed the strongest linkage evidence in this region among the sites. We carried out SNP fine-mapping of 4.36 Mb surrounding this linkage signal (1402 SNPs, average 3.1Kb density) on 103 of our 132 families that were most informative for 8p-linkage. Using 70 of these fine-mapping SNPs, selected to be pairwise uncorrelated (r^2<.05), we continued to see linkage (maximum NPL of 6.95 (p=2.95x10-10)) at rs11994515, in the center of the multi-site scan peak. Family-based association in these pedigrees showed modest support (p<0.05) for 13 of the 32 genes in the region, with the strongest signals for two SNPs 5 of ADRA1A, both with nominal p-values <0.001, and a concentration of 5 nominally significant SNPs in DPYSL2, a gene located ~100 kb telomeric of ADRA1A and previously associated in our family-based studies in the Ashkenazim (Liu et al 2008; Fallin et al., 2005). We performed an additional case-control study of a refined 2.8Mb section (25.7 - 28.5Mb, 23 known genes) using 970 SNPs (r^2.8) in 713 independent SZ/SZA cases and 1448 unrelated controls. Of the 970 SNPs, 12% showed nominal significance at p<0.05, and 3 SNPs showed a study-wide p<0.10 after permutations accommodating the multiple tests performed (rs6983735, rs6558061, rs4545109). Our strongest signal, located 13kb upstream of DPYSL2 at 26,476,491, achieved a nominal p = 0.000045 and study-wide p = 0.025. Further analyses by sex and SZ sub-phenotypes are now in progress.

Animal model of SPG6 hereditary spastic paraplegia. *Y. Y. Kisanuki[1], S. Rainier[1], J. Moore[1], T. Saunders[2], J. E. Wilkinson[3], J. K. Fink[1,4]* 1) University of Michigan, Neurology; 2) Internal Medicine; 3) Pathology; 4) Geriatric Research Education and Clinical Center, Ann Arbor Veterans Affair Medical Center, Ann Arbor, MI.

The hereditary spastic paraplegias (HSPs) are clinically and genetically heterogeneous disorders characterized by progressive lower extremity weakness and spasticity. SPG6 HSP is a particularly severe, autosomal dominant form of HSP. Our laboratory identified the SPG6 locus (15q11.1)and showed that *NIPA1* mutations cause SPG6 HSP. No treatments to stop, reverse, or prevent progressive disability are available. NIPA1 mutations are predicted to be pathogenic through gain of function because individuals with Prader-Willi/Angelman syndrome who have chromosome 15 deletions including the SPG6 locus do not develop progressive spastic paraplegia. NIPA1 mutations c.G316C and c.G316A, both resulting in p.G106R, are reported in SPG6 HSP.

We created transgenic rats in which the mutant human $NIPA1^{G106R}$ is expressed under the control of *Thy1.2* promoter. We analyzed 10 transgenic lines. These rats exhibit age-dependent (onset ~12 weeks), progressive hindlimb motor impairment. They exhibit hind limb clasping or extensor rigidity when lifted by their tails. Beginning at 16 to 20 weeks, gait becomes markedly abnormal with outwardly rotated hind limbs and scooting or hopping rather than stepping. They show difficulty in walking on rotating drum and narrow beam. Neuropathologic analysis reveals marked axonal degeneration in the spinal cord, particularly corticospinal and spinocerebellar fibers without primary myelin loss. Brain, muscle, and peripheral nerve do not show significant pathology. Spinal cord neuron cell bodies are preserved.

Behavioral and neuropathologic features of $NIPA1^{G106R}$ transgenic rats are parallel to those of human HSP. These animals are a valid model of SPG6 HSP and an important resource to explore its pathogenesis and treatment. Furthermore, our observations support the hypothesis that NIPA1 mutations are pathogenic through toxic gain of function mechanism.

Whole Genome Bisulphite Sequencing by High-Throughput Oligonucleotide Ligation Sequencing. *M. A. Barker[1], V. L. Boyd[1], Y. A. Sun[1], M. D. Rhodes[1], L. W. Jones[1], C. K. Monighetti[1], S. J. Stanley[1], V. I. Bashkirov[1], K. J. McKernan[2], F. M. De La Vega[1]* 1) Applied Biosystems 850 Lincoln Centre Drive Foster City, CA 94404; 2) Applied Biosystems 500 Cummings Center beverly MA 01915.

Methylation of CpG motifs throughout the genome plays a critical role in many cellular processes within the cell, including differentiation, chromosome stability, regulation of gene expression and preventing transcription of repetitive or alien sequences. Accordingly, aberrant methylation patterns have been linked to many diseases, and play a major role in the initiation and progression in the majority of cancers. Bisulphite sequencing is the method of choice for characterizing the methylation status of each CpG in a given region, however until recently bisulphite sequencing has been limited to region specific analysis. The introduction of next generation sequencing technologies removes this limitation, allowing hypothesis-free analysis of the entire methylome. Here we present a technique that harnesses the ultra-high throughput of the SOLiD System, a sequencing platform based on massively parallel cycled fluorescent oligonucleotide ligation and cleavage, with the single base level resolution of bisulphite sequencing, enabling genome wide methylation analysis. A novel adaptation to the fragment library protocol enabled the DNA library to be treated with bisulphite after the addition of sequencing adapters. The resulting bisulphite converted library was then subjected to emulsion PCR and sequencing using standard sequencing protocols. Alignment of the resulting reads to the reference genome present some challenges due to the length of the read and the reduction in sequence specificity after bisulphite conversion. Methods have been developed to address these problems. We believe the combination of gold standard bisulphite sequencing with the unprecedented throughput of next-generation sequencing removes long standing technical roadblocks to large scale methylation analysis at single base resolution, and will enable significant advances in the field.

A Genome-Wide Association Study (GWAS) for fractional exhaled nitric oxide (FeNO) levels in the Hutterites. *E. E. Thompson, S. Kuldanek, L. Pan, C. Ober* Department of Human Genetics, The University of Chicago, Chicago, IL.

Elevated levels of FeNO are a measure of airway inflammation. To date, no population-based studies have examined the heritability and genetic basis of FeNO. We measured FeNO, total serum IgE and lung function (FEV_1) and performed skin prick allergen tests (SPT) and studies of bronchial hyperresponsiveness (BHR) to methacholine in 561 Hutterites, a founder population of European descent. Levels of FeNO were significantly correlated with IgE ($P = 1.14 \times 10^{-6}$ after adjusting for age and sex) but not with FEV_1 ($P>0.1$) or BHR ($P>0.1$). FeNO was highly heritable ($H^2=1.0$, s.e.= 0.17) in the sample. We then performed a GWAS of ln FeNO using the Affymetrix GeneChip Mapping 500k Array. High quality genotypes for 295,307 autosomal SNPs with minor allele frequencies 0.05 were analyzed using a test of association that takes into account the relatedness between all pairs of Hutterites. Four SNPs met criteria for suggestive significance in this sample ($P<2.44 \times 10^{-5}$), including one on chromosome 11 upstream of a gene previously implicated in inflammation and Th cell differentiation ($P=3.56 \times 10^{-6}$), one on chromosome 2 ($P=2.26 \times 10^{-5}$) that is also associated with SPT in our sample ($P=0.007$), and a pair of SNPs in strong linkage disequilibrium also on chromosome 1 ($P<2.44 \times 10^{-5}$). The latter two SNPs are also associated with asthma in our sample ($P<0.037$). Here, we report the first GWAS for FeNO, a non-invasive marker of airway inflammation. We show that this trait is highly heritable and identify potential candidate genes influencing FeNO levels and, possibly, asthma and allergic phenotypes. Supported by NIH grant HL085197.

IL-6 cytokine pathway genes regulate cell death in inherited photoreceptor degenerations. *C. Jiang[1], M. Szego[1], L. Pacione[1], A. Miyajima[2], T. Hudson[3], R. Nadon[3], R. McInnes[1]* 1) Hosp for Sick Children, Toronto, Canada; 2) Univ of Tokyo, Tokyo, Japan; 3) McGill Univ, Montreal, Canada.

We previously reported that in inherited photoreceptor degenerations, the mutant photoreceptors (PRs) are at a constant risk of death (Clarke et al. Nature 2000). Using microarrays to identify genes that mediate the constant risk, we find up-regulation of a putative IL-6 cytokine pathway in 3 mutant PR mouse models, when 60-90% of PRs are still alive: in the $Rds^{+/-}$ model, *Oncostatin M* (2X up by qPCR)*Oncostatin M receptor* (*Osmr*)(2.6X up)*Stat3* (2.3X up)the transcription factor (TF) *C/EBP* (3.2X up), with increases in the cognate proteins Osmr (3X up), Stat3 (2.6X fold), and pStat3 (the active TF form of Stat-3), 5.8X up) (all $p<0.01$). These increases were predominantly in Müller glia, but the increased *C/EBP* mRNA was pan-retinal, including PRs. Since exogenous IL-6 cytokines have been shown to slow PR death and also increase Müller cell pStat3, we asked whether the *endogenous* increases in IL-6 pathway genes in mutant retinas were a survival response, and generated mutant PR models with *Osmr* or *C/EBP* loss of function (LOF) mutations. *Osmr* LOF decreased PR survival: 4 month old $Rds^{+/-}$;$Osmr^{-/-}$ retinas had 12.5% fewer PRs vs. *wt* (n=9, $p<0.05$), and 31 day-old *Tg(RHO P347S)*;$Osmr^{-/-}$ retinas had 13.5% fewer PRs (n=6, $p<0.01$). The putative IL-6 response pathway (above), if predominantly activated through the Osmr, should exhibit a decline in pStat3 levels in the absence of *Osmr*. Unexpectedly, *Osmr* LOF had no effect on pStat3 levels in $Rds^{+/-}$;$Osmr^{-/-}$ retinas, indicating that pStat3 activation is mediated through other IL-6 cytokines or other pathways. In contrast to the *Osmr* LOF, *C/EBP* LOF increased mutant PR survival:8-month old $Rds^{+/-}$;$C/EBP^{-/-}$ retinas had 18% more PRs vs. *wt* (n=5, $p<0.005$). We conclude that in mutant PRs 1) up-regulation of the *Osmr* is a survival response; 2) up-regulation of the *C/EBP* transcription factor is pathogenic, and therefore 3) *Osmr* and *C/EBP* act either in different pathways or different cells, in response to a PR mutation; 4) the partial effects of *Osmr* & *C/EBP* LOF indicate that other genes also mediate the constant risk of death.

Genome-wide detection and association of copy number aberrations in Late-Onset Alzheimer Disease. *GW. Beecham[1], ER. Martin[1], M. Slifer[1], J. Gilbert[1], J. Haines[2], M. Pericak-Vance[1]* 1) Miami Institute for Human Genomics, University of Miami, Miami, FL; 2) Center for Human Genetics Research, Vanderbilt University, Nashville, TN.

Late-Onset Alzheimer Disease (LOAD) has a strong but complex genetic component. One potential source for LOAD genetic risk that has received little attention is copy number variations (CNVs). To investigate the role of CNVs in LOAD, we evaluated multiple algorithms that use SNP genotype data for CNV detection and association, including Hidden Markov Models, and segmentation algorithms. Algorithms were evaluated using synthetic data with known deletions derived from X-linked data. We then applied the top performing tests to LOAD genome-wide association SNP intensity data from the Illumina 550k HumanHap beadchip. Promising regions were then tested for association using a permutation test. Additionally, single marker tests for differences in intensities were evaluated, including Students t-test, chi-square test, Mann-Whitney U test, and a mixture model for batch effect correction. Over twenty CNVs were significantly associated with LOAD using multiple algorithms. Many of the CNVs were under consensus linkage signals on 2q33, 6q12, 6q15, 10p12, 10q26, 12p11, 21q22, and 19q13 (unlinked to ApoE) and others were found at biological and locational candidate genes such as MAPT, CBS, SCARB1, ECHS1, PSMB7, and POMT1, many of which also have previously reported significant association results for LOAD. These regions should be targeted for further analysis, and their implication shows that CNVs likely play a role in LOAD risk that cannot be ignored.

Influence of Genomic Variance on the Transcriptome in Human Brain Tissues. *J. Gibbs[1,2], M. van der Brug[1], D. Hernandez[1,2], S. Lai[1,4], B. Traynor[1,3], M. Cookson[1], A. Singleton[1]* 1) Laboratory of Neurogenetics, National Institute on Aging, NIH, Bethesda, MD; 2) Department of Molecular Neuroscience and Reta Lila Weston Institute of Neurological Studies, IoN, UCL, London, UK; 3) Department of Neurology, John Hopkins University, Baltimore, MD, USA; 4) Department of Neurology, Chang Gung Memorial Hospital and College of Medicine, Taiwan.

Variation of gene expression effects development, function and risk of neurological disease within brain tissue. Multiple genomic sources contribute to variation of gene expression; including DNA sequence variation, genomic modifications such as methylation and expression of mircoRNAs (miRNA). The technologies are present now such that it is feasible to investigate the interrelatedness of genomic variance and the transcriptome from a whole genome perspective. The content and context of this data allows us to begin to interrogate genetic and epigenetic variation and their effects on transcript expression. Identifying the links between genomic variance and differences in expression, expression quantitative trait loci (eQTL), will facilitate our ability to begin to build functional maps of genomic regions within the human brain. Having such a map is useful not only for understanding genomic regions and the regulation of normal gene expression but also for association studies linking genomic loci with disease. In this study we have collected tissue from four brain regions (frontal and temporal cortex, cerebellum and pons) from 150 individuals. For each individual we genotyped 550,000 single nucleotide polymorphisms (SNPs) and in all four brain regions measured DNA methylation at 27,500 CpG islands, expression of 700 microRNAs (miRNAs) and 22,000 gene transcripts (mRNAs). Within each brain region analysis were performed to identify correlations by modeling pairings of different data types. Across regions analysis were performed to identify expression and methylation differences as well differences within the region specific correlations.

A weighted permutation method for efficient control of population structure. *K. Zhao[1], M. Nordborg[2], P. Marjoram[3], C. D. Bustamante[1]* 1) Department of Biological Statistics and Computational Biology, Cornell University, Ithaca, NY; 2) 2Molecular and Computational Biology, University of Southern California, Los Angeles, CA; 3) 3Division of Biostatistics, University of Southern California, Los Angeles,CA.

One of the main challenges in genome-wide association mapping is accounting for the confounding effects of population structure and cryptic relatedness. Since large sample sizes are often required for mapping complex traits, these confounding effects can be a significant source of spurious associations. Most current methods for solving this problem rely on inclusion of population structure estimates as covariates in ordinary least-squares regressions. However, for many complex pedigrees, these terms are not sufficient to account for all degrees of relatedness. Mixed model approach was effective in dealing with various levels of relatedness, but the normality assumption of phenotypic residual is usually not held. Here we presented a weighted permutation method, which improves upon the efficiency of these approaches. First, we estimated the relatedness between individuals from the genome-wide markers. We then perform a permutation test which weights phenotype by the relatedness matrix. By applying this method to various data sets ranging from Arabidopsis to dog to human, we show that our method effectively controlled the false positive rates. Using extensive simulations, we also demonstrate that our approach has very good power while controlling genome-wide false positives. Since our simulations vary many parameters simultaneously (e.g., causal SNP allele frequency, number of causal SNPs, the informativeness of population structure of causal SNPs, etc.), it provides one of the most thorough comparisons of existing approaches for controlling confounding effects of population structure and cryptic relatedness.

Inferential Genotyping in Mormon Founders and Utah pedigrees. *J. Gitschier* Dept Medicine & Inst Human Genetics, UCSF, San Francisco, CA.

One concern in human genetics research is maintaining the privacy of individuals who contribute samples for investigation. While this concern is raised typically in the context of private medical information, I would argue that a signficant contributor to loss of privacy may lie with genealogical investigations, as much information is freely available online through a variety of websites, thus facilitating the discovery of genetic relationships. During sabbatical in the laboratory of Chris Tyler-Smith (Wellcome Trust Sanger Center), I genotyped the Y chromosome of HapMap samples with 16 short tandem repeat (STR) markers as well as lineage specific markers to determine whether the Y chromosome genetic information in this sample was consonant with the purported ancestry of the subjects. As one of the HapMap populations (CEU) is comprised of Utah pedigrees of European descent, I then queried whether the contributors of these samples might be descendents of Joseph Smith and Brigham Young, two founders of the Latter-day Saints. Remarkably, through iterative use of two online archives, FamilySearch and Sorenson Molecular Genetic Foundation, I was able to infer the Y chromosome STR haplotypes of these two founders. Although none of the CEU contributors appeared to be direct descendents of the two men, based on haplotype analysis, I was able to make predictions for the surnames of the CEU participants by the same process. For more than half of the unrelated CEU samples (16/30), at least one exact match was revealed and for 13 of these, a single surname was associated. For the remaining 14 samples, a match was nearly perfect, with only one or two of the microsatellite markers varying, typically by only one repeat unit, as might be expected through microsatellite instability within a pedigree. By contributing samples and information to repositories specializing in genetic genealogy, individuals make important contributions to our collective knowledge, but they do so at the risk of revealing personal information shared by unwitting relatives. This problem will be exacerbated as genome-wide markers and sequences, which may bear physical, health and behavioral information, emerge and are employed in genealogical research.

Neuronal autophagy induction requires mTOR and protects against misfolded protein stress in inherited neurodegenerative disorders. *J. E. Young[1], R. A. Martinez[1], A. R. La Spada[1,2]* 1) Department of Laboratory Medicine, University of Washington, Seattle, WA; 2) Center for Neurogenetics & Neurotherapeutics, University of Washington, Seattle, WA.

Autophagy is emerging as a critical stress response pathway in many human diseases, including neurodegenerative ones. Despite the reported protective effects of autophagy, little is known about how neurons activate this integral response. To investigate the role of autophagy in neurodegeneration, we developed a primary neuron model of autophagy induction. Conversion of unconjugated LC3-I to phosphatidylethanolamine-conjugated LC3-II is a reliable method for assaying autophagy induction. The LC3-I to LC3-II conversion can be monitored by a change in subcellular distribution, going from a diffuse to punctate appearance when associated with autophagic vesicles, or, by observing a molecular weight shift upon Western blot analysis. To study neuronal autophagy, we cultured primary cortical neurons from transgenic mice that ubiquitously express GFP-tagged LC3. By subjecting GFP-LC3 neurons to systematic nutrient withdrawal, we documented robust autophagy activation, both by GFP-LC3 puncta formation and accumulation of LC3-II on immunoblots. Testing of various culture components allowed us to attribute neuronal autophagy induction to loss of the key growth factor insulin. Induction of autophagy, secondary to insulin deprivation, is dependent on mTOR inhibition, and could be abrogated by treatment with 3-methyladenine, or shRNA knock-down of beclin-1. To test if autophagy is induced by misfolded proteins in our primary neuron culture system, we expressed polyglutamine-expanded androgen receptor (polyQ-AR), the protein responsible for X-linked spinobulbar muscular atrophy, in primary cortical neurons from GFP-LC3 mice. Although polyQ-AR expressing neurons ultimately die an apoptotic death, autophagy was induced prior to cell death and significantly protected polyQ-AR expressing neurons from cell death. These results suggest that neurons are capable of inducing autophagy via a canonical pathway, and that autophagy can protect neurons from misfolded protein stress in inherited neurological diseases.

Association analysis of *NGFB* suggests a different genetic contribution to primary vs. substance-induced affective disorders. *DH. Cui[1,4], H. Zhang[1,4], H. R. Kranzler[5], H. P. Blumberg[1,4], A. R. Tyrka[6], BZ. Yang[1,4], F. Wei[1,4], L. L. Carpenter[6], L. H. Price[6], J. Gelernter[1,2,3,4]* 1) Dept Psychiatry, Yale Univ, West Haven, CT; 2) Dept Genetics, Yale Univ, School Med, New Haven, CT; 3) Dept Neurobiology, Yale Univ, School Med, New Haven, CT; 4) VA CT Healthcare Center, West Haven, CT; 5) Dept Psychiatry, Univ. of CT, School Med, Farmington, CT; 6) Dept. Psychiatry & Human Behavior, Brown Univ, Providence, RI.

Affective disorders (AFD), characterized by extremes of mood, are genetically influenced. We genotyped 1536 SNPs in or near 135 candidate genes in 868 European-American (EAs) and 348 African-American (AAs) using an Illumina GoldenGate Array. HelixTree software was used for association analysis. We focused on nerve growth factor (*NGFB*) gene. There was evidence for significant association to AFD of 4 of 15 SNPs genotyped in *NGFB* in EAs, and of 1 SNP in AAs. We re-analyzed these 15 SNPs using Haploview and FBAT software for case-control and family samples, respectively. Among 868 EAs, 372 were affected with AFD, including 182 with primary AFD (PAFD), 190 with AFD that was comorbid with drug dependence (CAFD), and 472 screened controls. Among 348 AAs, 164 were CAFD and 184 were controls. The family sample included 198 subjects in 77 EA families and 131 subjects in 49 AA families, with all affected subjects CAFD. 4 SNPs were associated with EA AFD (p=0.004-0.048). After stratification analysis, among EAs, 6 SNPs were significantly associated with PAFD (p=0.003-0.036), but none were associated with CAFD. In AA, 2 SNPs showed nominal association to CAFD (p=0.019 and 0.042). No markers were associated in the family sample. In haplotype analysis, these 15 markers formed 3 blocks in both the EA and AA populations. In EAs, TGG in block1 and AC and GT in Block2 were significantly associated with AFD (p=0.004-0.034). After stratification analysis, GG (p=0.0035) and AA (p=0.0035) in block3 were significantly associated with EA PAFD, as were TGG (p=0.037) and AC (p=0.0058). No haplotypes were significantly associated with CAFD in EAs or AAs. We conclude that *NGFB* is a risk gene for PAFD, but not for CAFD, suggesting that PAFD and CAFD have different genetic contributions.

Sequencing Candidate Genes for familial Thoracic Aortic Aneurysms and Dissections. *D. C. Guo[1], L. Wang[1], L. Li[1], N. Avidan[1], V. Tran-Fadulu[1], H. Pannu[2], S. Scherer[3], D. M. Milewicz[1]* 1) Dept Internal Medicine, Univ Texas/Houston Med Sch, Houston, TX; 2) Dept Nerosurgery, Univ Texas/Houston Med Sch, Houston, TX; 3) Hum Genome Center, Baylor College of Medicine, Houston, TX.

Thoracic aortic aneurysms leading to aortic dissections are inherited primarily in an autosomal dominant manner (FTAAD). Studies have determined that there is significant genetic heterogeneity for FTAAD; 4 genes have been identified through positional cloning that account for 20% of the disease. Mutations in genes for smooth muscle cell (SMC)-specific isoforms of two contractile proteins, alpha actin (ACTA) and beta-myosin (MYH11), are responsible for 14% and <1% of FTAAD, respectively. Since mutations in various components of the cardiac contractile unit lead to hypertrophic cardiomyopathy, we hypothesized that mutations in genes encoding other components of the SMC contractile unit may lead to familial TAAD. We have sequenced 16 candidate genes in 94 DNA samples from affected individuals from unrelated FTAAD families. DNA sequencing identified 24 novel nucleotide alterations in the coding regions of 12 genes. Sequencing of DNA from the probands families confirmed that 10 novel alterations in 8 genes segregate with TAAD in the families. These alterations are not present in 100 ethnically matched controls. Five alterations disrupted amino acids that are highly conserved and are predicted to disrupt the protein structure (http://genetics.bwh.harvard.edu/pph/). For example, a novel missense mutation alters the calmodulin binding domain of myosin light chain kinase (MYLK, S1759P). Phosphorylation of S1759 regulates MYLK activity by preventing calmodulin activation of MYLK and the mutation is predicted to similarly prevent calmodulin binding. These results support that hypothesis that genes encoding other components of the SMC contractile complex lead to FTAAD. Furthermore, this candidate gene approach has the potential to rapidly identify additional genes leading to FTAAD.

Pseudoaminopterin syndrome: case report with new characteristics. *M. Cernach, D. Brunoni, N. Sobreira, A. Perez*
Centro de Genetica Medica, UNIFESP-EPM, Sao Paulo, Brazil.

Fetuses exposed to aminopterin during the 8th-9th week of development may show aminopterin embryophathy (AE). Surviving children have a very specific phenotype that included unusual face, skull, and skeletal abnormalities. In 1987, Fraser et al. described 2 children with multiple malformations characteristic of the aminopterin syndrome but without any evidence of exposure to aminopterin in the mothers and suggested that this represents a new syndrome, the aminopterin syndrome-like sine aminopterin (ASSA) syndrome. Stevenson et al. (2004) described a male twin of a 40-year-old Hispanic mother born at 32 weeks of gestation with trisomy 9 and findings similar to pseudoaminopterin syndrome. It is also possible that a cryptic duplication involving part of chromosome 9 could contribute to the phenotype seen in patients with pseudoaminopterin syndrome. Here we describe a girl, 9 years old, born to unaffected first cousin parents. She has short stature, microcephaly, broad forehead with high hair implantation, central verticil; sparse and fine hair, areas of alopecia; arched eyebrows with upturned hair, synophris; ocular hypertelorism, epicanthal folds, palpebral ptosis; oligodontia; low-set and small ears with antihelixes hypoplasia; brachydactyly, bilateral clinodactyly of the 4th and 5th fingers; hypoplasia of the 4th metacarpal and clinodactyly of the 4th and 5th toes; overlap of the second over the third toe; bilateral hip luxation. Echocardiogram showed patent foramen ovale and pulmonary congenital disease. Abdominal ultrasound showed left posterior diaphragmatic hernia, absence of spleen and horseshoe kidney. She and her mother have a karyotype with 46, XX, inv9qh what have been described like a polymorphism, but, because of the possible involvement of chromosome 9 in ASSA, the CGH array will be done in this patient. Although this patient has some characteristics did not describe before in patients with ASSA syndrome, like: oligodentia,diaphragmatic hernia, absence of spleen, and horseshoe kidney, her phenotype suggest she has ASSA syndrome. However, we do not exclude the possibility that this is a different condition not described previously.

Altered Cytokine Signaling in Vascular Ehlers Danlos Syndrome (vEDS). *T. K. Cooper[1,3], Q. Zhong[1], U. Schwarze[2], M. Pepin[2], P. Byers[2], H. C. Dietz[3]* 1) Hershey Medical Ctr; 2) Univ of Wash; 3) Johns Hopkins.

vEDS is a severe connective tissue disorder caused by deficient type III collagen (Col3) due to intracellular retention and/or degradation of the mutant protein. Affected patients die prematurely due to rupture of arteries throughout the circulation. Historically, it has been inferred that disorders caused by mutations in connective tissue proteins manifest an obligate predisposition for tissue failure due to loss of structural integrity. This tenet has recently been challenged and refuted for Marfan syndrome (MFS), where a deficiency of fibrillin-1 leads to failed matrix regulation of TGF, and TGF antagonists can attenuate most manifestations in mouse models. Phenotypic overlap has been observed between vEDS and Loeys-Dietz syndrome, another vascular disease associated with increased TGF signaling. On this basis, we considered alternative pathogenetic mechanisms for vEDS. The Col3 N-terminal propeptide (Col3Npep) has curious properties including extreme evolutionary conservation, increased length for a signal peptide and apparent structure with 10 predictably spaced cysteine residues. Col3Npep shows substantial homology with the CR domains in chordin, an extracellular antagonist of TGF superfamily members, prominently the bone morphogenetic proteins (BMPs). Expression of recombinant Col3Npep in normal cells did not cause accumulation of pSmad2 (a mediator of TGF signaling) in response to TGF1 stimulation; this response was also normal in cultured vEDS fibroblasts. In contrast, expression of Col3Npep in control vascular smooth muscle cells reduced pSmad1/5 (a marker and mediator of BMP signaling) in response to BMP7. By extension, we hypothesized that vEDS cells that fail to secrete appropriate levels of Col3Npep would show increased BMP signaling. Indeed, cultured vEDS fibroblasts show increased accumulation of pSmad1/5 after pulse stimulation with BMP7 and at steady state when grown in the presence of 20% serum. We are currently analyzing a mouse model of vEDS and plan a therapeutic trial with a BMP antagonist. The propetide of other fibrillar collagens shares homology with chordin, highlighting the potential relevance to other collagenopathies.

The eMERGE Network: A national consortium of electronic health record-linked biobanks furthering large-scale genetic research. *W. A. Wolf[1], R. L. Chisholm[1], C. G. Chute[2], G. Jarvik[3], E. Larson[3], D. R. Masys[4], C. A. McCarty[5], D. M. Roden[4], J. P. Struewing[6]* 1) Northwestern University, Chicago, IL; 2) Mayo Clinic, Rochester, MN; 3) Group Health Cooperative/University of Washington, Seattle, WA; 4) Vanderbilt University, Nashville, TN; 5) Marshfield Clinic Research Foundation, Marshfield, WI; 6) National Human Genome Research Institute, Bethesda, MD.

Medical research institutions are developing biorepositories of genomic DNA coupled to electronic health record (EHR) information generated by routine clinical care. The goal of the Electronic Medical Records and Genomics (eMERGE) Network (www.gwas.net), organized by NHGRI in late 2007, is to investigate how such resources can be leveraged for genome and informatics science, with extensive ELSI input. Each of the five participating sites will use natural language processing or other tools to identify cases with defined phenotypes and controls for genome wide analyses in ~15,000 subjects, and will share data with the scientific community through NIHs dbGaP. The target phenotypes include asthma, type II diabetes, low HDL, cataracts, myocardial infarction, peripheral arterial and carotid disease, normal QRS duration, late life dementias (Alzheimers disease), and statin adverse events. Initial studies underway include testing computational algorithms to identify subjects meeting phenotype criteria, comparing these tools across diverse EHR systems, assessing the potential for combining cases or controls from different sites, and initiation of mechanisms to optimize community consultation. The goal of these studies is to contribute to our understanding of disease and develop recommendations to improve the utility of EHRs for research, setting the stage to integrate genomic and EHR data to achieve the vision and inform best practices for personalized medicine.

Genome-wide Association Study of Parkinson's disease by using 550K SNP Array. *W. Satake[1, 2], Y. Nakabayashi[1], I. Mizuta[1], T. Kawaguchi[3], T. Tsunoda[3], M. Kubo[3], S. Sakoda[2], M. Yamamoto[4], N. Hattori[5], M. Murata[6], Y. Nakamura[3], T. Gasser[7], A. B. Singleton[8], T. Toda[1]* 1) Div Clinical Genetics, Osaka Univ Grad Sch Med, Suita, Osaka, Japan; 2) Dept Neurol, Osaka Univ Grad Sch Med, Japan; 3) Cent Genomic Medicine, RIKEN, Japan; 4) Dept Neurol, Kagawa Prefectural Central Hosp, Japan; 5) Dept Neurol, Juntendo Univ Sch Med, Japan; 6) Dept Neurol, Musashi Hosp, NCNP, Japan; 7) Dept Neurodegenerative Diseases, Univ Tübingen, Germany; 8) Molecular Genetic Unit, Lab Neurogenetics, NIA/NIH, USA.

Parkinson's disease (PD), one of the most common neurodegenerative disease, is caused by multiple genetic and enviromental factors. We performed a Genome-Wide Association Study of PD, using Illumina HumanHap550 Array. Subjects were 1,012 PD patients and 2,573 controls (Sample call rate0.95) of Japanese ancestry. After SNP QC filter (a SNP call rate0.95, a MAF0.05, and HWE with a P0.001), high quality genotypes of 438,886 common SNPs were obtained (the average SNP call rate was 0.999). We excluded the samples with the cryptic duplicate and relatedness (MZ twin and 1-2 degree) through computing IBS probabilities, and the individuals who seemed to have non-Japanese ancestry were also excluded using multidimensional scaling. After these exclusions, 988 cases and 2,521 controls remained for the analysis. Genomic control method indicated that there was little evidence of any general inflation of the test statistics (genomic inflation factor = 1.05). We assessed each SNP for association with PD using a Cochran-Armitage trend test. A total of 127 SNPs were significant at the $P < 10^{-4}$. The most significant SNP was rs11931074 ($P=6.17 \times 10^{-13}$) and its neighbors, located in the 7 kb downstream - intron 4 of -synuclein. The second most significant locus included the EST on ch11q25 ($P=5.6 \times 10^{-7}$), which was newly identified as a potential susceptibility for PD. The MAPT locus was not identified significant in our study, because, unlike Caucasian, risk SNPs in the MAPT locus were monomorphic in the Japanese population, suggesting genetic heterogeneity of PD among races. Our finding will play an important role in clarification of the etiology of PD.

Identification of putative bipolar disorder susceptibility gene on chromosome 15q25-26. *J. A. Donald[1], E. Z. McAuley[2,3], J. M. Fullerton[2,3], I. P. Blair[3,4], P. B. Mitchell[5,6], P. R. Schofield[2,3]* 1) Dept Biological Sci, Macquarie Univ, Sydney, Australia; 2) Prince of Wales Medical Research Institute, Sydney, Australia; 3) Faculty of Medicine, University of New South Wales, Sydney, Australia; 4) ANZAC Research Institute, Sydney, Australia; 5) School of Psychiatry, University of New South Wales, Sydney, Australia; 6) Black Dog Institute, Prince of Wales Hospital, Sydney, Australia.

Previous linkage studies of mood and psychotic disorders have implicated a susceptibility locus on 15q25-26. We have also reported significant linkage to this region (two-point LOD 3.38 at D15S130) using 35 Australian multigenerational pedigrees affected with broad definition bipolar disorder (McAuley et al. Molecular Psychiatry, in press). Here we present the results of a positional association study to identify putative candidate genes under the linkage peak. We identified a 6.2Mb candidate region defined by the Zmax-1 linkage confidence interval and further mapped recombination breakpoints from family specific haplotypes to define a 2.7Mb high priority region. We selected a total of 376 SNPs at 20k resolution across the 6.2Mb interval, with increased resolution across the high priority region, for genotyping and association analysis in an Australian case-control cohort, comprising 187 cases and 199 controls. Association analysis identified 16 SNPs with uncorrected p 0.05, 13 of which lie in the high priority region, and 8 of which cluster in a 63kb window spanning exons 1-4 and including 16kb upstream of ST8SIA2. Haplotype analysis reveals a 6 SNP block which is overtransmitted to cases compared to controls (0.42 vs 0.32; global p = 0.034, haplotype specific p = 0.007). These data implicate ST8SIA2 as a putative bipolar susceptibility gene within the 15q25-26 linkage region. Interestingly, this gene has previously been reported in association with schizophrenia in Japanese and Chinese cohorts, and provides an attractive functional candidate which may constitute a general susceptibility factor to both schizophrenia and affective disorders.

Population Structure in Mongolia from a Mitochondrial DNA Perspective. *L. Pipes[1], A. A. Pai[2], D. Labuda[3], T. G. Schurr[2]* 1) Department of Biology, Swarthmore College, Swarthmore, PA; 2) Department of Anthropology, University of Pennsylvania, Philadelphia, PA; 3) Department of Pediatrics, University of Montreal, Quebec, Canada.

Mongolia has experienced a complex series of demographic movements over the past 10-20 millennia that have shaped the patterns of its modern human genetic variation. However, modern populations in Mongolia have not been extensively studied for DNA diversity, nor has the genetic contribution of Mongolians to the gene pools of contemporary populations in Southeast Asia and Oceania been fully resolved. Archaeological evidence from as early as the late Neolithic suggests the presence of both West and East Eurasian cultures in this region. Later demographic movements involving the emergence of the Mongolian and later Manchu Empires have further convoluted Mongolias population structure. To clarify the complex population history of Mongolia, we analyzed variation in the mtDNAs of 190 individuals from several Mongolian ethnic groups, including the Uriankhai, Zakhchin, Derbet, Khoton and Khalkha. We screened all samples for phylogenetically informative coding region SNPs and sequenced HVSI to assess control region variation in them. Our data suggest that the mtDNA diversity present in our population is consistent with the general pattern of variation observed in East Asia, with the most frequent haplogroups being C, D and G. Haplogroup variation in Mongolian ethnic groups reveals considerable maternal diversity with a predominance of basal M types. Interestingly, the Mongolians also possessed West Eurasian haplogroups, such as H, J and K, which are not commonly observed in East Asia, even at low frequencies. The main ethnic group in Mongolia, the Khalkha, was highly variable with respect to mtDNA haplotypes in comparison with the other ethnic groups, and clearly distinct from the Khoton and Zakhchin, as evidenced by distance measures. Overall, these data provide insights into the origins and affinities of these populations, their relationships with East Asian groups and neighboring Turkic speaking groups, including indigenous Altaians, and their possible role in the peopling of the Americas.

Genomewide associations with 10 nuclear magnetic resonance (NMR) subfractions of LDL, VLDL, and HDL among 12,000 Caucasian women from the WGHS. *D. Chasman[1], S. Mora[1], G. Paré[1], R. Zee[1], A. Parker[2], J. Miletich[2], P. Ridker[1]* 1) Preventive Med, Brigham & Women's Hosp, Boston, MA; 2) Amgen, Inc., Cambridge, MA.

While measurement of the standard clinical lipid fractions LDL, HDL, and triglycerides is invaluable for managing cardiovascular disease, measurement of subfractions of these lipoproteins by NMR reveals greater detail about lipid metabolism and may provide deeper insights into lipid-based cardiovascular risk. Specifically, NMR techniques can resolve LDL, HDL, and VLDL particles according to physical size (and thus density and composition), allowing determination of plasma concentration for four subfractions of LDL, as well as three subfractions each of HDL and VLDL. To better understand the full spectrum of genes and genetic variants influencing the NMR lipid subfractions, we performed a genomewide association study among 12,000 participants in the Womens Genome Health Study (WGHS) with verified Caucasian ancestry. The analysis revealed 23 loci at genomewide significance ($P5x10^{-8}$), almost all of which were consistent with current knowledge about lipid metabolism. Two loci, *FADS1/FADS2* (rs1535 for large LDL and HDL) and *PLTP* (rs6065906 for small HDL) were identified exclusively on the basis of genomewide associations with NMR subfractions and not with the conventional measurements of LDL, HDL, or triglycerides. An additional genomewide association in a gene-free region of 16p13.2 (rs1466405) with the NMR assay triglycerides appears to be entirely novel and was confirmed at sub-genomewide significance in the conventional measure of triglycerides. Our analysis further identified a broad range of association modes, with some variants exerting similar effects on several NMR subfractions at once (e.g. rs714052 at *MLXIPL* for subfractions of VLDL), others specifically influencing only one or two NMR subfractions (e.g. rs6709906 at *ABCG5/8* for large LDL), and still others having reciprocal effects on the NMR subfractions (e.g. rs5880, rs5883, and rs3764261 at *CETP* for subfractions of LDL and HDL). Thus, the study reveals highly detailed relationships among genetic determinants of plasma lipid subfractions as well as one novel associated locus.

SNP arrays in the cytogenomics lab: Telling us things we didnt know. *B. D. Thiel[1], L. K. Conlin[1,2], D. Dipatri[1], X. Gai[1], M. Xie[1], J. C. Perin[1], T. H. Shaikh[1,2], P. White[1], J. Glessner[1], C. Kim[1], L. M. Medne[1], C. Bonnemann[1], L. Campbell[1], D. Clark[1], I. D. Krantz[1], H. Hakonarson[1], N. B. Spinner[1,2]* 1) Children's Hospital of Philadelphia, Philadelphia, PA; 2) The University of Pennsylvania, Philadelphia, PA.

Array based platforms are now becoming standard in the cytogenetics laboratory due to their superb resolution for identification of genomic abnormalities. While CGH platforms have been the most frequently used to date, SNP genotyping arrays are proving to be outstanding for identifying abnormalities as well as providing mechanistic insights into complex rearrangements. We have validated the Illumina Human Quad610 SNP array for use in our clinical cytogenetics laboratory, detecting all of the known abnormalities (200 kb, 20 SNPs) in a test set of 117 patients with deletions or duplications. We also studied a group of 176 patients with unknown diagnoses and identified numerous abnormalities that were below the resolution of standard cytogenetics, including deletions, duplications, hidden mosaics, uniparental isodisomy, and patterns of meiotic crossing over that lead to complex chromosomal aberrations. We identified 55 variants (31%; 19 deletions/36 duplications), whose pathogenicity we could not completely predict (parental studies pending). We were also able to identify a change we believe is pathogenic in 17 patients (10%) including 10 deletions (6 novel,1 novel mosaic and 3 known), 5 duplications (2 novel, 3 known), and 2 complex rearrangements. One whole chromosome mosaic that had been missed by cytogenetic analysis (mos +14) was identified. In this case, genotype information revealed the mechanism as nondisjunction after crossing over in meiosis I. The most complex patient had XX and XY cell lines, and analysis of his genotype revealed him to be a parthenogenic chimera, with the XX cell line derived completely from the maternal genome. These unusual patterns of inheritance could only be identified using the intensity data in combination with the genotyping data offered on the SNP array platform. This work highlights the utility of the SNP array platform in both the clinical research cytogenetic laboratory.

Association between dopamine D4 receptor (*DRD4*) -521 C/T polymorphism and sensation seeking behavior in female alpine skiers and snowboarders. *C. J. Thomson[1], C. W. Hanna[2], P. Wang[1], K. L. Morton[1], M. R. Beauchamp[1], J. L. Rupert[1]* 1) School of Human Kinetics, University of British Columbia, Vancouver, British Columbia, Canada; 2) Department of Medical Genetics, University of British Columbia, Vancouver, British Columbia, Canada.

Previous research has shown a large genetic influence over personality traits, especially sensation seeking (SS). One gene thought to influence this behavioral trait is *DRD4*, in which variants have been associated with SS and novelty seeking in some, but not all studies. The inconsistencies between studies may be due to heterogeneity in both the behaviors and the populations being assessed. Some studies included only males and few studies have *a priori* analyzed males and females separately. SS has been associated with high-risk sports, including skiing; however this is the first study to address the possibility that genetics may play a role in individuals inclination towards SS in sport. Using the Contextual Sensation Seeking Questionnaire for Skiing (CSSQ-S), developed and validated for this study, and the Zuckerman-Kuhlman Personality Questionnaire (ZKPQ), levels of SS in males and females were analyzed in association with -521 C/T. Behavioral analysis of skiers (N = 201) revealed a significant correlation (r^2= 0.497, p 0.001) between skier behavior (CSSQ-S) and skier personality scores (ZKPQ). Preliminary genotype analysis (N = 50, ongoing) revealed allele frequencies of 0.52C and 0.48T. A significant association was found between the T allele (CT and TT genotypes) and low contextual skiing SS behavior in the females (N = 25, p = 0.04, ^{2} = 0.2), along with a non-significant trend between ZKPQ SS scores and -521 C/T (p = 0.06). No association, however, was found in males (N=25, p = 0.97). This study supports the hypothesis that alleles of the *DRD4* -521 C/T polymorphism are associated with context-specific SS behaviors in females, but not in males. Whether the lack of association in males is due to variations in the biochemical properties of the dopamine receptor or whether social pressures differentially influence male and female sensation-seeking behavior are areas that require further investigation.

Chromosomal Translocation t(5;7)(q14;q31) and Missense Mutations Implicate the Voltage-Gated Potassium Channel Kv4.2 gene, KCND2, on 7q31 in Autism. *A. Mikhailov[1], S. Choufani[2], J. Skaug[2], D. Kolozsvari[2], C. Marshall[2], S. W. Scherer[2], J. B. Vincent[1]* 1) Centre for Addiction and Mental Health, Toronto, Ontario, Canada; 2) The Hospital for Sick Children, Toronto, Canada.

Autism or autistic disorder (AD; MIM 209850) has a prevalence of 3-7/1000 among children. Evidence suggests that multiple interacting genetic factors are involved in AD, but the identity and the exact number of genes remain unknown. Numerous genome-wide linkage studies have suggested that chromosome 7q may harbour susceptibility gene or genes for autism and a number of reports have also identified AD patients with cytogenetic abnormalities mapping to this region. We have identified a male autism patient with a balanced de novo translocation t(5;7)(q14;q31). We have mapped the 5q and 7q translocation breakpoints using fluorescence in situ hybridization (FISH) and have identified BAC clones spanning both breakpoints. A patient with speech and language disorder and developmental delay with translocation t(2;7)(p23;q31.1), was reported by Lai et al, 2000, where the breakpoint maps within ~100Kb of the 7q breakpoint in our subject. For KCND2, the closest coding gene to these two 7q31 breakpoints, an amino acid sequence variant N544S was identified in one proband.Upon further direct sequencing analysis of about a 1000 unrelated autism probands, two additional sequence variants,F538S and R539L, were identified in two unrelated probands. It is notable that all three sequence variants are within a region of 5 amino acids and might indicate an important functional region. *KCND2* encodes the brain-specific voltage-gated potassium channel Kv4.2 (Shal-related). Kv4.2 is critical in controlling the excitability of neurons, and is regulated at the synapse by PSD-95. Other synaptic proteins have already been implicated in autism, such as neuroligins 3 and 4, neurexin 1, SHANK3, also dipeptidyl-peptidase-like proteins DPP6 and DPP10. Interestingly, Kv4.2 forms complexes with DPP6 and DPP10 to generate neuronal subthreshold A-type channels. These important findings implicate the KCND2 gene in autism and extend our understanding of a neurobiological pathway involved in the etiology of autism.

Mitochondrial D-loop genetic variation is associated with frailty in Caucasians from the Cardiovascular Health Study (CHS). *S. McGuire[1], M. L. Biggs[2], A. L. Rea[1], A. O'Connor[1], B. Beamer[3], M. D. Fallin[4], A. Chakravarti[1], L. P. Fried[5], J. Walston[3], D. E. Arking[1]* 1) Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD; 2) Dept of Biostatistics, University of Washington Sch Public Health and Community Medicine, Seattle, WA; 3) Div of Geriatric Medicine and Gerontology, Johns Hopkins University Sch Med, Baltimore, MD; 4) Bloomberg Sch of Public Health, Johns Hopkins University, Baltimore, MD; 5) Mailman Sch Public Health, Columbia University Medical Center, New York, NY.

Frailty is a geriatric syndrome characterized by reduced muscle strength, mobility and physical activity, and vulnerability to adverse health outcomes. A compelling case can be made that dysregulation of energy metabolism, whose key components are encoded by the mitochondrial genome, can lead to tissue function decline, and ultimately phenotypic changes that may underlie frailty. To investigate whether inherited genetic variation in the mitochondrial genome is associated with frailty, we fully resequenced mtDNA from 157 younger frail cases (mean age=77.8, 42 males and 115 females) and 157 older robust controls (mean age=81.9, 41 males and 116 females) drawn from CHS, a longitudinal study of adults 65 years and older designed to identify risk factors and outcomes of cardiovascular disease. Frailty was defined as the presence of 3 of 5 frailty components (weakness, slowness, weight loss, exhaustion, and low physical activity) at baseline. Participants were excluded based on the presence of confounding medical conditions, including stroke, Parkinsons disease, or dementia, and given the limited number of non-Caucasians they were also excluded. Only 1 SNP, mt204 in the D-loop, was found to be significantly associated with frailty after multiple test correction using permutation (P0.001). In sex-stratified analyses, we identified a male-specific association for mt146 (P0.003) and female-specific association for mt228 (P0.041). These results indicate that mitochondrial variation may have an impact on frailty susceptibility in Caucasian populations, and involvement of the D-loop suggests that the mechanism may involve altered mitochondrial replication efficiency.

Regulation of ATM recruitment to damaged DNA. *P. Bradshaw[1], M. S. Meyn[1,2,3]* 1) Prog Genetics & Genome Biol, Hospital for Sick Children, Toronto, ON, Canada; 2) Dept Molecular Genetics, Univ of Toronto, Toronto, ON, Canada; 3) Dept Paediatrics, Univ of Toronto, Toronto, ON, Canada.

ATM, the protein kinase deficient in the cancer predisposition syndrome ataxia-telangiectasia, controls the major DNA double strand break (DSB) repair pathway in human cells. Accumulation of MRN-activated ATM protein at DSB sites leads to local phosphorylation of ATM target proteins and creation of regions of modified chromatin that serve as scaffolds for repair proteins. In order to better understand ATMs role in the DNA damage response, we generated DSBs in predefined nuclear regions of human fibroblasts with UVA light + Hoechst dye. ATM is detected at photo-induced DNA damage as early as sixty seconds post damage and prior to the DNA damage response factors H2AX and 53BP1. Unlike H2AX, ATM does not persist at damage sites, as maximal accumulation of ATM occurs between ten and thirty minutes post damage. We find that ATM displays tight spatial localization with damaged DNA, in contrast to progressively diffuse foci formed by Mre11, H2AX, 53BP1 and Rif1. Consistent with this observation, FRAP data indicates that GFP-tagged ATM is stably associated with DNA damage sites. Accumulation of both endogenous ATM and GFP-ATM at DNA damage sites is undetectable in cells deficient in Mre11, a component of MRN. In contrast, mutating the ATM 1981 phosphorylation site from serine to alanine impaired but did not block accumulation of GFP-ATM. Further, over-expression of the telomeric protein TRF2, but not the related protein TRF1, attenuates GFP-ATM accumulation at DNA damage sites and impairs phosphorylation of ATM, H2AX and p53. Our data indicate that ATM differs from many DNA damage response proteins in its rapid, localized association with damaged DNA, behavior consistent with idea that ATM stably associates with DSBs. ATM recruitment to damage sites requires functional MRN, while optimal accumulation of ATM at damaged DNA entails phosphorylation at serine 1981. In contrast, interactions with TRF2 dampen the ATM response to DSBs, suggesting TRF2/ATM interactions promote local repair of DNA damage while inhibiting ATM-dependent global DNA damage responses.

A family study using a novel ascertainment strategy replicates locus linked to plasma Factor XII activity on 5q33 and identifies novel loci on 8q24 and 1p36. *F. Gagnon[1], G. Antoni[1], Y. Luo[1], A. Tuite[1], D. Bulman[2], P. Wells[2]* 1) School of Public Health, Univ Toronto; 2) Ottawa Health Research Institute, Canada.

We took advantage of a common and well-characterized major gene variant associated with venous thromboembolism (VTE), i.e. Factor V Leiden (F5L), to localize Quantitative Trait (QT) loci underlying variation in plasma Factor XII activity (FXII). FXII is correlated to VTE and has emerged as a promising therapeutic target with reduced bleeding risk. Identifying FXII QT loci will provide clues as to the mechanisms underlying VTE, as well as better risk profiling opportunities for its prevention. We ascertained five extended French-Canadian families on a proband not only having a history of VTE, but also carrying the major gene variant F5L, with the goal of reducing genetic and clinical heterogeneity; and measured ~30 hemostatic/lipid QT, along with environmental covariate data, in all members (n=267). We did a genome-wide oligogenic linkage analysis (1079 microsatellites) of FXII, based on Bayesian MCMC methods and covariate adjustments. We provide evidence for 3 QT loci located on chromosome (ch) 5, 8 and 1, with posterior probabilities of linkage (computed as Intensity Ratios in 2 cM interval) of 240, 87 and 25, respectively. The ch5 locus corresponds to the F12 gene, which has been implicated in variation of FXII in the GAIT (Genetic Analysis of Idiopathic Thrombophilia) study. The ch8 (8q24) and ch1 (1p36) loci are novel. Adjusting for anti-thrombotic treatment did not affect the signal on ch8 but decreased by ~ 50% the signals on ch5 and ch1. Adding F5L in the model did not modify the linkage signals on ch5 and ch1 but reduced it by ~35% on ch8, suggesting that the latter locus interacts with F5L. We are investigating pleiotropic effects with other QT/VTE, as well as epistasis. In conclusion, using innovative ascertainment and analytic strategies, we localized strong linkage signals for FXII, demonstrating that it is possible to detect linkage with a relatively small but carefully selected sample. Attempts to replicate the loci will be done in two independent French samples with complementary designs.

SNP allele differentiation provides independent confirmation of genomic imbalance. *J. H. Tepperberg, I. Gadi, B. Williford, V. Jaswaney, J. Kesler, P. R. Papenhausen* Dept Cytogenetics, Laboratory Corp America, Res Triangle Pk, NC.

The whole genome 1.8 million feature SNP/copy number microarray (Affymetrix, Inc.) provides high-resolution detection of DNA copy number changes in individuals with idiopathic mental retardation and developmental delay. One of the unique features of the SNP based microarray is the specific SNP allele difference confirmation of DNA copy number changes and designation of copy neutral homozygosity correlated with UPD and consanguinity. The SNP allele call is not dependent on copy number changes and therefore provides a secondary confirmation of genomic aberrations. The specific allele designation was found to be essential for the precise identification of true gain and loss and base pair boundary determination required for precise genotype-phenotype correlation. This polymorphic nucleotide allele difference provides confirmation by identifying regions of loss of heterozygosity associated with deletions, allele specific dosage gain associated with duplications, and long contiguous stretches of homozygosity (LCSH) associated with UPD and consanguinity. For example, only one SNP allele possibility exists for all targets within a deletion interval or within regions of homozygosity (i.e., no heterozygosity, only alleles A or B). The absence of heterozygosity becomes confirmation of the deletion segment. Four possible SNP genotype clusters are generated across duplicated intervals (AAA, AAB, ABB, BBB). The dosage ratio of these genotypes provides confirmation of the duplicated chromosomal segment. Three SNP clusters (AA, AB, BB) would indicate the absence of a true duplication. The SNP allele dosage differentiation is thus a powerful independent confirmation of chromosome copy number aberrations and reduces the need for secondary follow-up in the proband. We will present characteristic patterns of allele specific copy number analyses of microdeletions (including nullosomy X), duplications and copy neutral LOH associated with LCSH. Further applications would be to use the SNP allele dosage percentage to evaluate constitutional low-level mosaicism and DNA amplification in oncology.

NRG1 SNPs associated with the schizophrenia phenotype in Hispanic Populations. *H. Raventos[1], J. M. Peralta[2], C. Walss-Bass[3], A. Ontiveros[4], H. Nicolini[5], R. Mendoza[6], L. Almasy[2], M. Escamilla[3]* 1) CIBCM, Univ Costa Rica, San Pedro, San Jose, Costa Rica; 2) Soutwest Foundation for Biomedical Research, San Antonio, TX; 3) Department of Psychiatry, University of Texas Health Science Center, San Antonio, TX; 4) Center of Investigation, School of Medicine, INFOSAME, Monterey, Mexico; 5) Medical and Family Research Group, Carraci S. C, Mexico DF; 6) Department of Psychiatry, David Geffen School of Medicine at UCLA, Torrence, CA.

The neuregulin 1 gene (NRG1) represents one of the most promising candidate genes associated with schizophrenia (SC). The evidence comes both from positive linkage findings on chromosome 8p12-p21 and association studies in several populations including Iceland, Scotland, the United Kingdom, China, Japan and Costa Rica. In this multi-centric schizophrenia study, we analyzed 662 Hispanic multiplex families, recruited from the Southwest USA, Mexico and Costa Rica. Patients were diagnosed by a consensus best estimation process based on DSMIV criteria. Two different affected/unknown phenotypes were constructed for analysis based on best estimated diagnosis: SC (n=472) and PHIST, patients with psychosis history regardless of their primary diagnosis (n=684). A total of 69 SNPs covering the ~1MB of the NRG1 gene were typed by Illumina with a custom SNP array. Association analyses were conducted on both, SC and PHIST, phenotypes with the 69 SNPs targeting NRG1 using FBAT under the assumption of an additive biallelic model. We found strong association between SC and rs10093683 (|z|=2.633, p=0.008) and in a lesser degree with rs3924999 (|z|=2.062,p=0.039), rs12547858 (|z|=2.180, p=0.029), rs13259346 (|z|=2.104, p=0.035) and rs2439322 (|z|=2.046, p=0.041). Also, rs10093683 (|z|=2.152, p=0.031) together with rs7812718 (|z|=2.334, p=0.020) were slightly suggestive of association with PHIST. None of these results are significant after a Bonferroni correction for the non-independence of the tests (=0.004). However, with a less conservative RFDR (=0.025) some of the results are still significant.

Whole Population, Genomewide Mapping of Hidden Relatedness. *A. Gusev[1], J. K. Lowe[2,3,4], M. Stoffel[5], M. J. Daly[3,6,7], D. Altshuler[3,4,7,8], J. L. Breslow[2], J. M. Friedman[2,9], I. Pe'er[1]* 1) Columbia University, NY, NY; 2) The Rockefeller University, NY, NY; 3) Broad Institute, Cambridge, MA; 4) Dept of Molecular Bio, MGH, Boston, MA; 5) ETH Zurich, Zurich, Switzerland; 6) Ctr for Human Gen Res, MGH, Boston, MA; 7) Dept of Medicine, HMS, Boston, MA; 8) Dept of Genetics, HMS, Boston, MA; 9) Howard Hughes Med Institute, Chevy Chase, MD.

Identifying and quantifying the sharing of genetic material between individuals within a population is an important step in accurately using genealogical relationships for disease analysis as well as improving our understanding of demography. However, exhaustive pairwise analysis which has been successful in small cohorts cannot keep up with the current torrent of genotype data. We present GERMLINE, a robust algorithm for identifying pairwise segmental sharing which scales linearly with the number of input individuals. Our approach is based on a dictionary of haplotypes, used to efficiently discover short exact matches between individuals and then expand these matches to identify long, nearly-identical segmental sharing that is indicative of relatedness. We use GERMLINE to survey hidden relatedness both in the HapMap and in a densely typed island population of 3,000 individuals. We verify that GERMLINE is in concordance with other methods when they can process the data, and also facilitates analysis of larger scale studies. We also demonstrate novel applications of precise analysis of hidden relatedness to detection of haplotype phasing errors and structural variation. We show that shared segment discovery can help identify phasing errors and potentially resolve them. Additionally, we use detected identity of genomic segments to expose polymorphic deletions that are otherwise challenging to detect, with 8/14 deletions in the HapMap samples and 153/200 deletions in the island data having independent experimental validation. Reinforcing the potential for a population-based approach to linkage analysis, we have successfully begun applying GERMLINE to larger and more out bred populations, as well as to quantitative trait mapping using shared haplotypes - identifying a novel haplotype associated with an increase in plant sterol levels.

Identifying Balanced Translocation Embryos and Normal Embryos, using combine method of FISH and Polymorphic Markers in PGD analysis. *A. Aviram-Goldring, J. Shamash, H. Resnik-Wolf, J. Dor, J. Levron, M. Dekel, E. Pras, S. Rienstein* Danek Gertner Institute of Human Genetics, Sheba Medical Center,Tel Hashomer, 52621 Tel Aviv, Israel.

Purpose: Our goal was to develop an improved PGD method by combining FISH and segregation mode of polymorphic markers. By using those methods, we can identify balanced, unbalanced and chromosomally normal embryos, and avoid implantation of affected embryos due to unbalanced translocation. Methods: IVF, embryo biopsy and PGD using FISH with different probe combinations, provided the possibility of identification the embryos. Special polymorphic markers, located close to the specific translocation breakpoint, were selected for segregation analysis of the blastomers. Results: A 25 year old female, carrier of Robertsonian translocation 45,XX, t(15;21)(p10;p10) attended our reproductive center for PGD. She had experienced two selective terminations of trisomy 21 pregnancies and three spontaneous abortions. She underwent IVF followed PGD using FISH with probes for chromosomes 15 and 21 and a pregnancy was achieved. Amniocentesis revealed that the embryo carried the balanced translocation. UPD was excluded and a healthy child was born. Two years later, she delivered another healthy normal child using the same procedures. With parents consent, segregation mode of polymorphic markers was analyzed in DNA extracts from their blood, amniotic fluid of the balanced translocated embryo, and blastomers derived from remaining abnormal embryos in order to determine the translocation status. Fourteen polymorphic markers within chromosome 15 and 21 were applied to identify the balanced translocated embryos and the normal karyotype. Conclusion: Our preliminary results indicate that the polymorphic markers segregation method is capable of distinguishing between normal, balanced, unbalanced translocation embryos. A larger cohort of chromosomal carriers should be investigated in order to improve the crucial steps in single cell DNA amplification. By using this method we might eliminate infertility and other problems that might affect balanced translocation carriers in the next generation.

Copy number variation screening with the 500K SNP array in patients with mental retardation. *K. Kobayashi[1], T. Mure[1,2], K. Kaneshiro[1], T. Chiyonobu[1,4], A. Nishimura[3], K. Hirai[5], M. Morimoto[3], M. Matsuo[2], J. Inazawa[6], T. Toda[1]*
1) Division of Clinical Genetics, Department of Human Genetics, Osaka University Graduate School of Medicine, Osaka, Japan; 2) Department of Pediatrics, Kobe University Graduate School of Medicine, Kobe, Japan; 3) Department of Pediatrics, Kyoto Prefectural University of Medicine, Kyoto, Japan; 4) Department of Pediatrics, Akashi Municipal Hospital, Akashi, Japan; 5) Department of Pediatrics, Kyoto Prefectural Disabled Children's Development Support Center, Kyoto, Japan; 6) Department of Molecular Cytogenetics, Tokyo Medical and Dental University, Tokyo, Japan.

The genetic factors underlying mental retardation (MR) are very heterogeneous. Recent studies have identified a number of genes involved in MR, but the current understanding of the monogenic causes of MR is far from complete. Investigation of chromosomal rearrangements in patients with MR has proven particularly informative in the search for novel causative genes. In this study we used the Affymetrix 500K SNP array together with the GEMCA software to screen for novel pathogenic copy number variations (CNVs) in 27 children with unexplained mental retardation. After excluding the regions which overlapped with reported CNVs seen in normal individuals, we found 16 CNVs in ten patients as candidates for the causative regions of MR. They include seven duplications and nine deletions ranging in size from 47 kb to 16 Mb, which were all confirmed by qPCR. Three of the candidate CNVs overlapped with known deletion or duplication syndromes, namely 22q11.2 deletion syndrome, Sotos syndrome, and Potocki-Lupski syndrome. Of the 13 remaining candidate CNVs, one is a 16 Mb deletion on chromosome 7q33-q36.1. The others remain to be further elucidated in order to identify causative genes of MR. The 500K SNP array is a powerful tool for the genetic analysis of patients with MR.

Mutations in GDF6 Associated with Klippel-Feil Syndrome Types I-III. *R. Clarke[1], M. Tassabehji[2], Z. Zhao[3], E. Hilton[4], J. McGaughran[5], E. Howard[6], M. Malass[2], D. Donnai[2], A. Diwan[7], F. Manson[4], Z.-M. Fang[1]* 1) St George Hosp, Sch Medicine, Univ of NSW, Kogarah, Australia; 2) Academic Unit of Medical Genetics, University of Manchester, St Mary's Hospital, Hathersage Road, Manchester M13 0JH, UK; 3) Department of Psychiatry and Human Genetics and Center for the Study of Biological Complexity, Virginia Commonwealth University, Richmond, Virginia 23298, USA; 4) Centre for Molecular Medicine, University of Manchester, Manchester, UK; 5) Queensland Clinical Genetics Service, Royal Children's Hospital and Health District, Herston Road, Herston , Brisbane 4029, Queensland, Australia; 6) National Genetics Reference Laboratory, St. Mary's Hospital, Manchester, UK; 7) Spine Service, St George Hospital, Kogarah, NSW 2217, Australia.

Klippel-Feil syndrome (KFS) is best characterized by congenital fusion of cervical vertebrae and is of unknown aetiology. KFS is characterised by genetic heterogeneity, with autosomal dominant, recessive and sporadic forms, and a great degree of clinical variability even within affected families. We identified a chromosomal inversion inv(8)(q22.2q23.3) segregating in a large KFS family with a unique autosomal dominant form of KFS associated with carpal/tarsal fusion and severe vocal impairment. Chromosome analyses localised the proximal inversion breakpoint downstream of GDF6, a member of the bone morphogenetic protein (BMP) family implicated in vertebral development and carpal/tarsal fusion in Gdf6 mouse models. GDF6 missense mutations identified in both familial and sporadic cases of KFS confirmed the role of GDF6 in KFS (Types I-III). The discrete patterns of Gdf6 expression during development show precise positional correspondence with the fusions and associated anomalies displayed in both KFS families and in knockout mice. Phenotypic gradients also appear to implicate GDF6s role as an extracellular morphogen. The staggering range of developmental phenotypes indicate a role for GDF6 in development of joints, cartilage, bone, tendon, nerve, kidney, spine, hand, foot, ear, face, mouth, throat and larynx development with important roles in posture, facial structure, dexterity, hearing, mirror movements and vocal development.

***PAX2* Novel Missense Mutation Causes Optic Nerve Colobomas of Variable Severity.** *H. Feret[1], E. Pierce[2], M. Bower[3], X. Wang[3], M. Falk[1]* 1) Human Genetics, CHOP, Philadelphia, PA; 2) Ophthalmology, CHOP/UPENN, Philadelphia, PA; 3) Genetics & Metabolism, U Minnesota, Minneapolis, MN.

PAX2 mutations cause autosomal dominant renal-coloboma syndrome or isolated optic nerve colobomas, with reduced penetrance and variable expressivity. We report a kindred with a *PAX2* novel missense mutation. Five individuals have optic colobomas and/or morning glory syndrome of varying severity, including several asymptomatic individuals. The proband is a 13-year-old girl with bilateral optic nerve colobomas with pits, history of tracking difficulties and nystagmus, 20/25-30 visual acuity, nyctalopia, headaches, post-pubertal obesity, severe infections, cervical kyphosis, and mild dysmorphism. One sister has mild bilateral morning glory syndrome with increased optic nerve size and abnormal retinal blood vessel pattern only on funduscopic photograph, normal vision, and resolved strabismus; one sister has abnormal optic nerves with large excavated cups and unusual vascular problems and 20/40 visual acuity; a brother has asymmetric optic nerve colobomas, impaired visual acuity, esotropia, and astigmatism. The father has asymmetric optic nerves and amblyopia. All have photophobia. Additional findings include learning/behavior problems and delays, headaches, hypotonia, failure to thrive, mitral valve prolapse, absence seizures, hypertelorism, and recurrent anaphylaxis. Paternal grandfather had kidney and bladder cancer. *PAX2* analysis in the proband revealed a c.250G>A transition resulting in an exon 3 Glycine to Serine substitution (G84S). Mutation confirmation in affected family members is underway. Almost all reported *PAX2* mutations result in a truncated PAX2 protein. Two other missense mutations have been reported, and both reside in the conserved paired box domain. One of these results in a similar Glycine to Serine substitution (G76S), in a kindred with a three generation history of kidney failure and mild or asymptomatic optic disc anomalies (Devriendt et al., Hum Genet 1998). These cases suggest *PAX2* mutation analysis be considered in cases of AD optic nerve colobomas/morning glory syndrome, regardless of renal manifestations or symptomatic visual impairment.

Keratin 1 (KRT1), a gene prioritized by age-correlated transcriptional profiling of 1,240 individuals, exhibits SNP genotype x age interaction in genome-wide association. *J. W.Jr. Kent[1], T. D. Dyer[1], H. H. H. Goring[1], J. Charlesworth[1], V. P. Diego[1], J. E. Curran[1], M. P. Johnson[1], M. Carless[1], J. B. M. Jowett[2], M. C. Mahaney[1], L. Almasy[1], J. W. MacCluer[1], E. K. Moses[1], J. Blangero[1], S. Williams-Blangero[1]* 1) Dept. of Genetics, Southwest Foundation for Biomedical Research, San Antonio, TX; 2) International Diabetes Institute, Caulfield, Victoria, Australia.

Individual humans age at different rates, but the causes of heritable variation in biological aging are largely unknown. Microarray expression profiling allows direct investigation of differences in gene expression with age. Göring et al. (Nat Genet 2007; 39:1208-1216) examined genome-wide gene expression in lymphocytes from 1,240 Mexican Americans in the San Antonio Family Heart Study (SAFHS) and identified 750 autosomally-encoded transcripts that are cis-regulated, i.e., their expression quantitative trait loci map to their structural genes. Here we have identified a subset of 30 validated, cis-regulated autosomal transcripts that exhibit significant (5% false discovery rate) correlation with age and polygenic genotype by age (GxA) interaction. Within this subset, a transcript of the gene keratin 1 (KRT1) ranks highest by joint evidence for cis-regulation, age correlation, and GxA. KRT1 encodes an important structural component of the spinous and granular layers of epidermis, and haplotype variants have been associated with variation in wound healing, a process that is profoundly impacted by aging. Of 34 haplotype-tagging SNPs at the KRT1/1B locus genotyped in 858 SAFHS individuals, 23 show genome-wide significant evidence of association ($P<1.3x10-7$) with KRT1 expression. 11 show locus-wide evidence ($P<0.0015$) of SNP genotype x age interaction (SxA), including rs2741158 (association: P=9.3 x10-52; SxA, P=0.0002) and rs939607 (association: P=1.3 x10-27; SxA, P=8.8 x10-5). These results demonstrate the efficacy of a multidimensional approach to gene prioritization, and identify KRT1 as a target for molecular dissection of the mechanisms of differential aging.

Genome wide copy number polymorphism study of sporadic Parkinson disease. *D. Malhhotra[1], S. Yoon[1], D. Hernandez[2], P. Abou-Sleiman[3], S. McCarthy[1], V. Makarov[1], J. Kendall[1], A. Leotta[1], A. Bhandari[1], A. Singleton[2], J. Sebat[1]* 1) Cold Spring Harbor Laboratory, Cold spring harbor, NY; 2) NIA Porter Neuroscience Research Center, Bethesda, MD-20892; 3) Institute of Neurology, University College London, UK.

We performed a two staged genetic association study to investigate the hypothesis that copy number variants (CNVs) comprise a significant proportion of genetic risk factors for sporadic Parkinson disease (PD). In Stage I, we used a cross platform CNV validation approach by individually scanning genomes of 159 late onset Caucasian PD cases, 151 early onset PD cases and 228 matched control samples on two CNV discovery platforms, i.e 85K genome wide ROMA (Representational Oligonucleotide Microarray Analysis) and 550K Illumina Hapmap Single Nucleotide Polymorphism (SNP) Chip. We found evidence for several novel rare recurrent and non recurrent CNVs in PD cases which might play an important role in disease pathophysiology. Among the most compelling findings from Stage I cross platform validated novel rare recurrent CNVs include i) a partial duplication of gene, PARD3 in 2 late onset PD cases ii) deletion of autophagy gene ATG12L, in 2 early onset PD cases and iii.) a duplication involving exons 5-8 of Parkin (PARK2) gene in 2 siblings with early onset PD in a family with history of PD. We also tested association of 181 accurately genotyped common CNVs with risk of sporadic PD but did not detect any significant association. In Stage II, genome wide CNV scans of an additional independent cohort of 431 late-onset sporadic Caucasian PD cases and 365 matched Caucasian controls were performed using Illumina 550K SNP chip. Interestingly, we observed a rare duplication of another autophagy gene, ATG5 in a sporadic late onset PD case. Autophagy genes play an important role in controlling neurodegeneration and our results provide new evidence for their role in PD. Our large scale CNV study of sporadic PD cases and controls has identified several potentially interesting new candidate genes which could be important in etiology of PD.

Overlapping risk loci for bipolar disorder and schizophrenia: analysis of 40 dopaminergic pathway genes. *V. Nimgaonkar[1,2], M. E. Talkowski[1], H. Mansour[1], J. Wood[1], L. McClain[1], K. Prasad[1], D. Montrose[1], K. Chowdari[1], D. Axelson[1], B. Birmaher[1], D. Lewis[1], E. Frank[1], D. Kupfer[1], T. Monk[1], S. Faraone[2], N. Laird[2], J. Smoller[2], M. Thase[2], P. Sklar[2], B. Devlin[1,2]* 1) Departments of Psychiatry, Neurobiology, and Human Genetics, University of Pittsburgh, Pittsburgh, PA; 2) Systematic Treatment Enhancement Program for Bipolar Disorder (STEP-BD) Consortium.

Background: A shared genetic etiology for bipolar disorder (BP1) and schizophrenia (SZ) / schizoaffective disorder (SZA) has been proposed but never comprehensively tested. Motivated by previous associations and epistatic interactions between dopaminergic (DA) genes and SZ, we evaluated the hypothesis of overlapping risk loci for BP1 and SZ/SZA from the DA pathway.

Methods: We tested 431'tag' SNPs ($r^2 < 0.9$) chosen to represent all publicly avialable common variations from 40 DA genes simultaneously in both disorders. Our cohorts included 526 BP1 cases, 531 SZ/SZA cases, and 477 screened adult controls.

Results: Gene-based tests suggested a common risk factor for both disorders, the Dopamine D3 receptor gene (*DRD3*), following Bonferroni correction. Disorder specific tests were noteworthy for BP1 at *DDC*, *DRD1IP*, and *FREQ*; *DRD2* was associated with SZ/SZA. Analyses of individual SNPs yielded overlapping associations for these disorders at 15 SNPs, representing 60% of all nominally significant SZ associations and 41% of all BP1 associations ($p < 0.05$ in both disorders).

Conclusion: Our results suggest shared DA risk factors for BP1 and SZ/SZA, as well as disorder specific associations. Replicate studies are required. *Additional co-authors from affiliation #2: M. Allen, C. Bowden, J. Calabrese, R. El-Mallakh, A. Fagiolini, M. Fossey, E. Friedman, L. Gyulai, P. Hauser, T. Ketter, J. Loftis, L. Marangell, D. Miklowitz, A. Nierenberg, J. Patel, G. Sachs*.

Variation in the Atrogin-1 Gene (FBXO32) in Patients Experiencing Statin-Induced Severe Adverse Events. *A. M. K. Brown, V. Normand, Y. Renaud, J.-C. Tardif, M. S. Phillips* Genome Quebec and Montreal Heart Institute Pharmacogenomics Centre, Montreal, QC, Canada.

Statins are among the most highly prescribed medications in the world. One of the most common adverse events observed in patients taking statins is muscle toxicity that can range from isolated pain to life-threatening rhabdomyolysis. Recently, Hanai et al. (JCI. 2007) tested the hypothesis that the Atrogin-1, a muscle specific protein highly expressed during atrophy, may be involved in a patients sensitivity to statins. They observed that Atrogin-1 expression was significantly higher in patients experiencing muscle pain while on statins and showed that muscle cell damage increased in both muscle cells in culture and zebrafish upon addition of statins. As part of a large Genome Canada project, we are currently in the midst of collecting a cohort of 2,500 patients experiencing statin-induced adverse events (myalgia, severe myotoxicity, and rhabdomyolysis) and 2,500 controls (patients receiving statin therapy without muscle-related adverse reaction). With access to our unique cohort, we have decided to test Hanai et als observation in humans experiencing statin induced adverse events to determine if underlying variation within the Atrogin-1 gene (FBXO32) differs between cases and controls. In order to test this hypothesis, we have sequenced the FBXO32 gene in 96 selected patients experiencing severe statin-induced muscle toxicity. The sequencing reactions included all coding regions plus 1kb upstream and downstream for both isoforms of the gene. In total, 71 SNPs and 1 microsatellite have been observed. Forty of the SNPs are novel, and have not been publicly reported as of build 128 of dbSNP. We are now screening these observed variations in our control population to see if there are any associations that can be made. To this end, we have developed genotyping assays on the Sequenom MassArray platform to screen for all 72 variations in our populations. The resulting association analysis, using both single SNPs and haplotypes, will show if variation within the FBXO32 plays a role in the pathogenesis of statin-induced muscle toxicity.

Most L1 retrotransposition events occur in embryogenesis and are not inherited. *H. Kano[1], E. M. Ostertag[2], I. Godoy[1], G. L. Gerton[3], T. Merdiushev[3], H. H. Kazazian Jr.[1]* 1) Dept Genetics, Univ Pennsylvania, Philadelphia, PA; 2) Transposagen Biopharmaceuticals Inc. Philadelphia, PA; 3) Center for Research on Reproduction and Womens Health, Univ Pennsylvania, Philadelphia, PA.

L1s are abundant retrotransposons that comprise ~17% of the human genome. Despite having a great impact on the genome, relatively little is understood about L1 biology. However, because the genome is littered with L1s, the prevailing view has been that L1 retrotransposition occurs nearly exclusively in germ cells. Indeed, several studies of L1 transgenic mice have demonstrated that L1 retrotransposition can occur in germ cells, but also in the early embryo, using L1s driven by a heterologous promoter, not the natural L1 promoter. To characterize definitively the timing of human L1 retrotransposition, we studied transgenic mouse and rat models harboring a highly-active human L1 under the control of its endogenous promoter. L1 insertions were seen in more than 60% of the transgene containing mouse offspring and 100% of transgene containing rat offspring. In addition, L1 insertions were found in 5-10% of mouse and rat offspring lacking the transgene. All de novo L1 insertions observed in offspring were somatic, because they were rarely if ever inherited by the next generation. These non-heritable L1 insertions were seen even in mice and rats without L1 transgene, suggesting that L1 RNA can be carried over from ova and sperm and integrate into the genome during embryogenesis. In fact, as further evidence of RNA carryover we found L1 RNA and L1 insertions in individual blastocysts that lacked L1 transgene. We also found abundant L1 RNA from the L1 transgene in spermatogenic cells but relatively weak evidence of L1 insertion. On the other hand, L1 retrotransposition events were much more apparent in embryos than in spermatogenic cells. Thus, it appears that most L1 retrotransposition events occur in embryogenesis, suggesting a role for L1 insertions in human development and disease. That L1 RNA present in one generation can alter the somatic DNA of a subsequent generation is another example of the non-canonical role RNA can play in determining the human phenotype.

Childhood Acute Lymphocytic Leukemia: A Cytogenetic and FISH Perspective. *T. K. Jansz, G. Shikora, R. Kazi, J. Jacob, A. Gruia, M. Nouri, J. Mo, D. Assenza, C. Bowman, A. Aviram-Goldring* Cytogenetis, North Shore Long Island Jewish Medical Center, New Hyde Park , NY.

Acute Lymphocytic leukemia (ALL), the most common form of cancer in children is an important area of research and clinical study. Clonal proliferation of these hematopoetic cells is often the result of genetic abnormalities. It is known that the molecular presentation of such abnormalities, frequently chromosomal translocations, has a strong correlation to both the mechanism and prognosis of ALL treatment. The purpose of this study is to compare commonly accepted genetic aberrations of ALL in literature with the abnormalities observed in the community served by the Long Island Jewish Medical Center. Data for regional, gender, and chromosomal abnormalities were analyzed for trends. Sixty-four ALL patients, with ages from 1 to 20yrs were analyzed during 2006-2008. Twenty bone marrow karyotypes were analyzed from each patient. FISH was also performed using BCR/ABL, TEL/AML1, MLL, 4, 5, 7, 8,9,10,17 and inv(16) probes (Abbott Molecular,IL). Of the 64 patients, 16 had normal karyotypes (25%), and 48 had abnormal karyotypes (75%). Of the total patients, 26(40.6%) indicated a hyperdiploid state, 28(43.4%) had abnormalities in BCR/ABL, TEL/AML, MLL, or other translocations, 8(12.5%) had deletions in chromosomes, and 4(6.25%) had other chromosomal abnormalities. Increase in abnormal karyotypes were seen in Queens, Nassau, Suffolk, Bronx and Brooklyn in descending order of prevalence. Although our data reaffirmed the validity of some of the predicted prevalence of the accepted researched texts, an increased prevalence of hyperdiploidy and the TEL/AML translocation were observed in our community. The data collected revealed some significant regional trends with Queens and Nassau, NY as giving rise to the most pediatric patients presenting with ALL as well. This study emphasized the value of analyzing community data and its contributions in genetic analysis for the diagnosis and treatment outcomes of childhood acute lymphocytic leukemia.

LD-based SNP discovery and genotype calling from DNA sequence data. *P. Scheet* Center for Statistical Genetics, Dept. of Biostatistics, University of Michigan, Ann Arbor, MI.

The advent of high-throughput DNA sequencing technologies promises to allow detailed surveys of human genetic variation. However, current sequencing technologies are prone to nontrivial error rates. Furthermore, the depth of sequenced reads for a given individual is not uniform and may leave some loci with sparse coverage of mapped reads. These factors contribute to difficulties in calling individual genotypes or verifying whether individual loci are polymorphic. The dependence of alleles at nearby loci (linkage disequilibrium; LD) provides a sort of built-in redundancy of sequenced loci. Here, I adapt a model for LD among tightly-linked SNP markers to DNA sequence data for the purpose of explicitly taking account of this dependence, thereby allowing one to "borrow strength" from nearby markers when making single-marker inferences, where read depth may be sparse. Since quality scores associated with individual reads contain information about the accuracies of the estimated alleles, I fit the model directly to likelihoods for the true genotypes to account for uncertainties in the allele calls, and compare the utility of this approach to fitting the model to the allelic counts or called genotypes. In addition, by allowing for multiple alleles at each site, including a null allele, I can accommodate the presence of tri-allelic SNPs and short deletions segregating in the population. I apply the method to simulated data as well as to data from The 1000 Genomes Project to demonstrate that the incorporation of LD information leads to an improvement in polymorphism discovery and obtaining accurate probabilities of individual genotypes. These methods are implemented in the software package fastPHASE.

Gene set analysis of bipolar disorder using whole-genome SNP data. *Y. Meng[1,2], M. Ogdie[1,2], M. Ferreira[1,2], H. Gurling[3], D. Blackwood[4], A. Corvin[5], N. Craddock[6], P. Sklar[1,2], S. Purcell[1,2]* 1) Center for Human Genetic Research, Massachusetts General Hospital, Boston, USA; 2) Stanley Center for Psychiatric Research, Broad Institute of Harvard and MIT, Cambridge, USA; 3) Department of Mental Health Sciences, Windeyer Institute of Medical Sciences, University College London, UK; 4) Division of Psychiatry, University of Edinburgh, UK; 5) Trinity Centre for Health Sciences, St James's Hospital, Trinity College Dublin, UK; 6) Department of Psychological Medicine, Cardiff University, UK.

Most current approaches to whole-genome association studies (WGAS) test each variant sequentially and without regard to the genetic context. Although this comprehensive and agnostic approach has advantages, when high degrees of allelic and locus heterogeneity exist, alternative analytic strategies might perform better. Bipolar disorder is a complex genetic disease that likely displays just such high levels of heterogeneity. We recently performed a meta analysis [Ferreira et al.] of three independent GWASs of bipolar disorder, which supported association with the alpha-1C subunit of the L-type voltage-gated calcium channel gene, *CACNA1C* (combined P = 7.0 × 10-8, rs1006737). *CACNA1C* is one of 27 genes known to code for the calcium ion channel. To more deeply test the hypothesis that ion channel dysfunction is critical in bipolar disorder, we leveraged the coverage of whole-genome data to interrogate variation in this entire set of functionally-related genes, containing over 900 SNPs. The 27 genes are grouped according to subunit (coded A1, A2D4, B and G) and A1 subtype (P/Q, N, L, R and T). We applied a multi-locus set-based test at the level of pathway, subunit, subtype and gene. The results indicated a strong specificity of L-type alpha 1 subunit genes (P=0.0003) (with *CACNA1D* and *CACNA1S* showing association, P=0.019 and 0.002). Removing *CACNA1C*, the pathway, A1 subunit and L-type genes remained significant (P=0.0024 and 0.003 respectively). These results provide convergent support for *CACNA1C*: the genes are functionally related but statistically independent in terms of variation. We further discuss the methodological strategies to gene-based and pathway-based association analysis.

Intellectual Property Challenges for Development of Multi-gene Diagnostic Tests. *S. Chandrasekharan, R. Cook-Deegan* GELP, Duke University, Durham, NC.

Advances in technologies for sequencing and genotyping make it possible to test for hundreds of genetic variations and mutations simultaneously. Such diagnostics could potentially be faster, more comprehensive and cost effective and are well suited to evolving clinical and research needs, especially as additional mutations continue to be discovered. However, the development of chip or microarray based multi-gene diagnostics faces several challenges. One obstacle may be a complex intellectual property (IP) landscape. Difficulty in securing freedom to operate could prove a major impediment to the development of such promising diagnostics. To better understand the effects of patents and licensing on the development of such new tests, we studied genetic testing for three conditions, hearing impairment, spinocerebellar ataxia and Long QT syndrome (LQTS), which are characterized by mutations in multiple genes. We created intellectual property landscapes by identifying patents that cover relevant gene sequences, or mutations/variants and methods for diagnosing these syndromes. We also searched the scientific literature and patent databases to identify chip based diagnostics for these conditions that may be potentially developed for clinical testing. We identified several pending patent applications on microarray diagnostics for hearing loss and LQTS and other channelopathies that contain overlapping claims and include all patented mutations. We also identified licensing data for relevant patents from publicly available resources and interviews conducted with experts. Our preliminary analysis provides evidence for potential IP barriers to the development of such multigene tests. They include possible blocking effects of exclusively licensed patents for common gene mutations (hearing loss and Long QT), increased royalty payments for multiple licenses required from many different owners (SCA testing) and difficulty in assembling IP required for comprehensive testing of all alleles because of dominant positions of exclusive licensees (SCA, LQTS). Further studies will involve detailed analysis of claims to assess scope of patents and if potential uses in multi gene chips are precluded.

A large-scale high-density linkage study of autism identifies multiple genome-wide significant loci. *D. E. Arking[1], L. A. Weiss[2], C. W. Brune[3], K. West[1], E. H. Cook[3], M. J. Daly[2], A. Chakravarti[1]* 1) Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD; 2) Broad Institute of Harvard and MIT, Cambridge MA; 3) Institute for Juvenile Research, University of Illinois at Chicago, Chicago, IL.

Autism is a childhood neuropsychiatric disorder that, despite high heritability, has largely eluded efforts to identify genetic variants underlying its etiology. Using Affymetrix arrays we genotyped ~4,000 samples comprising 802 affected sib-pairs (ASPs) for ~500,000 SNPs. Samples were obtained from the NIMH Autism Genetics Initiative and AGRE, with each family having at least 1 child diagnosed with autism and a second child classified as autism, broad spectrum, or Not Quite Autism. We performed two genetic studies: a family-based linkage analysis (this abstract) to search for loci that harbor multiple, likely rare, susceptibility alleles, and, an association analysis to examine the role of common variants (Weiss et al., this meeting). For linkage analysis, we removed SNPs with frequency 20%, 1% missing data, 1 Mendelian errors, or in Hardy-Weinberg disequilibrium (P0.001) resulting in ~180,000 high-quality SNPs. Non-parametric ASP linkage was performed using MERLIN with linkage disequilibrium (LD) modeled by clustering SNPs with pair-wise r^2 0.1, revealing 4 significant (lod 3.6) peaks at 6q25.2, 15q25.3, 17p12, 17q11.2, and 1 highly significant (lod=5.7) peak at 6q27. To verify that these results were not due to artifacts introduced by clustering or from SNPs in high LD, we removed all SNPs with pair-wise r^2 0.2, and observed no significant change in linkage peaks. We also performed analyses using every other SNP, and again, observed no significant change, indicating that these results are unlikely to be influenced by nearby LD or poor genotyping assays. Aside from 15q25.3 and 17q11.2, these loci show little overlap with previous reports, and none of the loci overlap with suggestive regions of association. In summary, we have used high-density SNP genotyping to identify 5 genome-wide significant loci that contribute to autism susceptibility, and likely harbor rare variants exhibiting allelic heterogeneity.

Genome Wide Association Studies of Angiographic Coronary Artery Disease. *MP. Reilly[1], M. Li[1], MS. Burnett[2], A. Qasim[1], NN. Mehta[1], S. Restine[1], M. Wolfe[1], A. Edmondson[1], I. Stylianou[1], J. Devaney[2], B. Keating[1], T. Cappola[1], H. Hakonarson[1], Z. Chen[1], C. Knouff[3], V. Mooser[3], SE. Epstein[2], DJ. Rader[1], WTCCC, MI-GEN Consortium, Ottawa Heart Study, ADVANCE, Intermountain Heart Study, Emory CV Gene Bank* 1) Univ of Pennsylvania Medical Center; 2) Cardiovascular Research Institute/MRI/Washington Hospital Center; 3) GlaxoSmithKline.

Recent genome wide association studies (GWAS) identified a few novel loci for coronary artery disease (CAD), in particular at 9p21. However, no GWAS have been reported where CAD cases & controls were classified using gold-standard coronary angiography. We performed combined analysis of two case-control GWAS of angiographic CAD in Caucasians (Affymetrix 6.0; cases had >50% stenosis 1 vessel; controls w/ normal angiography): PennCATH (N=1401; 470 MI-CAD acute cases, 463 non-MI CAD chronic cases, 468 controls); Medstar (N=1322; 421 MI-CAD, 454 non MI-CAD, 447 controls). Sample call rates were >95% (mean 98%). SNPs studied had MAF >1%, were in HWE (p>10-7), with call rates >95%. There was no significant population stratification (0.99-1.03). Age & gender adjusted analyses of genotyped (PLINK) and imputed (MACH imputed and SNPTest association) SNPs included all CAD cases vs. controls & subgroups of MI/non MI cases vs. controls. Analysis replicated the 9p21 locus (best SNP vs. controls; all CAD (P = 1.2x10-8), MI-CAD (P = 8.9x10-10), non MI-CAD (P = 4.4x10-4). Several novel SNPs with CAD associations p<1x10-7 were identified. Stage 2 in silico replications (SNP p<10-3) are ongoing in 5 independent CAD GWAS (7,000 cases) with stage 3 genotyping (meta-analysis SNPs p<5x10-8) in 3 more angiographic CAD studies (5,000 cases). Conclusion: Initial findings suggest that our angiographic CAD GWAS replicates findings in clinical CAD GWAS, but also will identify novel CAD loci. An angiographic phenotype may identify loci related to coronary atherosclerosis itself (atherosclerosis locus), or those related to acute clinical complications only (plaque rupture/thrombosis locus).

Familial Thoracic Aortic Aneurysms and Dissections: A Subset of Families with Thoracic Aortic Aneurysms in Men and Intracranial Aneurysms in Women. *S. K. Medrek[1], V. Tran-Fadulu[1], A. C. Braverman[2], D. M. Milewicz[1]* 1) Dept Internal Medicine, Univ Texas/Houston Med Sch, Houston, TX; 2) Dept Internal Medicine, Washington Univ School of Medicine, St Louis, MO.

Thoracic aortic aneurysms leading to type A dissections are usually inherited as an autosomal dominant condition with variable expression and decreased penetrance (FTAAD). Genetic heterogeneity for FTAAD is established and correlates with clinical heterogeneity. For example, FTAAD resulting from TGFBR1 or TGFBR2 mutations is associated with aneurysms and dissections of other arteries, including fusiform intracranial aneurysms. In our cohort of 450 families with FTAAD, we noted that approximately 10% of families had one or more members with intracranial berry aneurysms (ICA), in contrast to the fusiform aneurysms associated with TGFBR1/2 mutations. In three large families, multiple women with ICAs were confirmed to harbor the defective gene causing TAAD based on their location in the pedigree. Interestingly, males in these families tended to have TAAD and females tended to have ICA. Linkage analysis with DNA from the largest family with 12 affected members indicated that the phenotype was not linked to any of the known TAAD loci and no TGFBR1/2 mutations were identified, indicating that the subphenotype of TAAD/ICA is caused by a novel gene. Imaging of both the thoracic aortic and intracranial arteries is recommended in these families. Detection of a novel gene would increase the mechanistic and clinical understanding of both TAAD and ICA.

Hermansky-Pudlak Syndrome (HPS) as a genetic model for Idiopathic Pulmonary Fibrosis (IPF): The role of MUC1 in pulmonary fibrosis. *T. Markello[1], B. Pederson[1], M. Anahtar[1], G. Srivastava[1], K. O'Brien[2], B. Gocchicco[1], W. Gahl[1]* 1) Section on Human Biochemical Genetics, Medical Genetics Branch NHGRI, Bethesda, MD; 2) Intramural Program of the Office of Rare Diseases, OD, NIH, Bethesda MD.

The 8 subtypes of HPS include occulocutaneous albinism and a platelet bleeding disorder. Genetic subtypes 1 and 4 also exhibit fatal pulmonary fibrosis(PF), making them models for the interstitial lung disease, IPF. MUC1, a membrane glycoprotein associated with invasive epithelial cancers and interstitial lung diseases, is a component of regenerating type II pneumocytes. These cells have lamellar bodies which are lysosome-like organelles resembling those affected in HPS. We propose that MUC1 is mislocalized in HPS type II pneumocytes. Indeed, using blood from HPS patients archived over the past 15 years, we see a strong correlation between MUC1 serum levels (up to 20 times the upper limit of normal) and degree of fibrosis (decline in pulmonary function) among 100 HPS individuals. No overlap is seen between normal and HPS (with fibrosis). Normal blood level of MUC1(mean)= 120U/ml (range 109-150,n=8). HPS patients without PF = 250U/ml(60-572,n=46). HPS patients with moderate PF = 590U/ml(299-2079,n=33). HPS patients with severe PF = 2141U/ml(743-10165,n=21). We demonstrated that sustained elevation of serum MUC1 in 21 HPS patients predicted present or future pulmonary fibrosis and was correlated with disease progression. A previous and current therapeutic trial of HPS fibrosis investigated pirfenidone, a TGF-beta inhibitor that functions through the SMAD signaling pathway. One HPS patient receiving pirfenidone had 3 episodes of MUC1 elevation over 10 years of treatment, but always returned to normal, presumably because of pirfenidones inhibition of SMAD signaling. This patient exhibited no lung disease progression over those 10 years, a remarkable finding. We conclude that: 1) MUC1 serves as a biomarker for disease prediction and as an outcome parameter for therapy. 2) Pirfenidone dampens the fibrotic effects of TGF-beta stimulation and reduces the amount of MUC1 produced in the interstitium. 3) HPS serves as a genetically homogeneous model in which to study the therapy of pulmonary fibrosis.

Genome-wide scan for linkage to type 1 diabetes in 2,496 multiplex families from the Type 1 Diabetes Genetics Consortium. *W.-M. Chen[1,2], P. Concannon[1,3], C. Julier[4], G. Morahan[5], B. Akolkar[6], H. A. Erlich[7], J. E. Hilner[8], J. Nerup[9], C. Nierras[10], F. Pociot[9], J. A. Todd[11], S. S. Rich[1,2], Type 1 Diabetes Genetics Consortium* 1) Ctr Public Hlth Genomics, Univ Virginia, VA; 2) Dept Public Hlth Sciences, Univ Virginia, VA; 3) Biochemistry & molecular Genetics, Univ Virginia, VA; 4) Inst de Genomique CNG, France; 5) Ctr for Diabetes Research, WAIMR & Ctr Medical Research, Univ Western Australia, Australia; 6) Div Diabetes, Endocrinology and Metabolic Diseases, NIDDK, NIH, MD; 7) Roche Molecular Systems, CA; 8) PHS, Wake Forest Univ, NC; 9) Steno Diabetes Ctr, Denmark; 10) JDRF, NY; 11) JDRF/WT DIL, Cambridge Inst for Medical Research, Univ Cambridge, UK.

Type 1 diabetes (T1D) is a complex disorder arising from the combined actions of multiple genetic and environmental risk factors. Recent genome-wide association (GWA) studies have identified a number of common genetic variants that contribute to T1D risk; however, these studies have limited power to detect loci where multiple rare alleles contribute to T1D risk. To identify rare genetic variants that are linked to T1D, the Type 1 Diabetes Genetics Consortium (T1DGC) has assembled a large collection of multiplex T1D families consisting of 2,496 families with 2,881 affected sib pairs (ASPs) from 9 geographic regions. We report the results of a genome-wide scan for linkage using 6,090 SNPs in the T1DGC family collection. Evidence for linkage to T1D was detected in the HLA region (6p21.3), the insulin locus on chromosome 11p15.5, a region on 2q33 containing CTLA4, and a region on distal 6q (6q27), with LOD scores 215.3, 3.1, 3.3 and 5.2 respectively. To incorporate the potential heterogeneity effect of sex or age-onset on T1D, we utilized a model in which IBD sharing from an ASP was regressed on the probability distribution that is defined by the ASPs covariate values. This regression-based covariate analysis allowed us to identify an additional region on chromosome 19p13.3 with $p=8\times10^{-5}$. Our analysis also provided evidence of potential linkage to T1D with p.001 at additional regions on chromosomes 2q13, 10q21.1, 18p11.22, 19q13.11, and 19q13.4.

A Comparison of Approaches to Account for Uncertainty in Analysis of Imputed Genotypes. *J. Zheng, Y. Li, G. R. Abecasis, P. Scheet* Center for Statistical Genetics, Department of Biostatistics, University of Michigan, Ann Arbor, MI.

The recent availability of a high-density reference panel, such as "The HapMap", has allowed for the imputation of genotypes at single nucleotide polymorphism (SNP) markers that were untyped in a cohort or case-control study but that have been characterized in the reference panel. These imputation procedures, which are based on patterns of linkage disequilibrium (dependence of alleles at nearby SNPs; LD) allow direct testing of these SNPs in the study sample of individuals. A natural question from such a procedure is how best to take account of the uncertainty of the imputed genotypes. We simulated a set of genotype data to mimic that from realistic cohort-based association studies and quantitative trait values for genetic models with varying degrees of dominance, independently at 83,327 SNPs. After imputation, we obtained the conditional probabilities of each possible genotype at untyped SNPs. Here, we compare the performance of the following 3 strategies for dealing with the results from imputation: regression on the "best-guess" imputed genotype; regression on the allelic or genotypic "dosage"; and the use of mixtures of regression models. We consider a range of minor allele frequencies and imputation accuracies to compare the power of the different methods under multiple genetic models. We find that the "best-guess", which does not accommodate the uncertainty in the imputed genotype, performs the worst, although only when the imputation accuracies suffer. Only in specialized situations, with large genetic effects and low imputation accuracies, does the mixture model does give greater power than using the allelic dosage. We therefore conclude that for the most realistic settings for current genome-wide association studies, regressing the phenotype on the estimated allelic or genotypic dosage provides an attractive compromise between accuracy and computational tractability.

An epigenetically demarcated chromatin insulator at a boundary between X chromosome inactivated and expressed transcripts of the UBE1 gene. *K. E. Prothero, L. Carrel* Penn State College of Medicine, Hershey, PA.

In females, X chromosome inactivation silences most genes on one X chromosome. Nonetheless ~15% of human genes escape inactivation, and are expressed from both active (Xa) and inactive (Xi) Xs. Many escape genes cluster and are likely coordinately regulated. CTCF-bound insulators have been found at several boundaries between inactivated and escape genes, but their role in Xi expression is unclear. To further examine insulators in Xi expression and identify additional Xi regulatory sequences, we focused on the human escape gene, UBE1. We established that UBE1 has a distinctive gene structure and Xi expression pattern; two alternative 5 untranslated (UTR) exons are X inactivated whereas 2.1 kb downstream another alternative 5 UTR exon escapes inactivation. Eight overlapping constructs within the human UBE1 boundary region were tested for insulator function and a 330 bp sequence with enhancer blocking activity was identified. The expression boundary is also epigenetically marked; by bisulfite sequencing, the region is unmethylated on the Xi but heavily methylated on the Xa. Intriguingly, the orthologous mouse gene has similar gene structure, but all transcripts are X inactivated. Therefore, sequences regulating Xi expression should be absent at the mouse Ube1x locus. Indeed, the human UBE1 boundary region shares high sequence similarity with mouse Ube1x, except for a 600 bp region that includes the enhancer blocking activity. Further, all mouse Ube1x sequences tested lack insulator activity and were heavily methylated on the Xi. These data suggest proteins involved in human UBE1 Xi regulation will specifically bind the unmethylated boundary on the Xi. Intriguingly, CTCF consensus binding sites are not found in the insulator. Nevertheless, we are testing CTCF binding by chromatin immunoprecipitation and will determine whether this region utilizes novel CTCF-binding sites or whether Xi regulation involves additional proteins that bind insulator sequences. This small expression boundary at UBE1, together with the human and mouse genetic and epigenetic differences makes this an ideal locus to evaluate factors regulating Xi expression.

Genome-wide association for insulin resistance and secretion in 542 genotyped and imputed individuals. *F. Xiang[1], L. J. Scott[1], Y. Li[1], A. U. Jackson[1], C. J. Willer[1], H. M. Stringham[1], M. R. Erdos[2], L. L. Bonnycastle[2], K. Kubalanza[2], A. J. Swift[2], G. R. Abecasis[1], K. L. Mohlke[3], J. Tuomilehto[4], R. N. Bergman[5], F. S. Collins[2], R. M. Watanabe[5], M. Boehnke[1]* 1) Biostatistics, U Michigan, Ann Arbor, MI; 2) NHGRI, Bethesda, MD; 3) Genetics, U North Carolina, Chapel Hill, NC; 4) Nat'l Public Health Inst., Helsinki, Finland; 5) Physiology and Biophysics, Keck School of Medicine, U Southern Cal, Los Angeles, CA.

Understanding the genetic variation that underlies regulation of insulin secretion and resistance and glucose levels will enhance our understanding of glucose homeostasis and type II diabetes. Here, we seek to test for association of genetic variants with parameters from the frequently sampled intravenous glucose tolerance test (FSIGT): acute insulin response (AIR), disposition index (DI), insulin sensitivity (S_i), and glucose effectiveness (S_g). We analyzed FSIGT data on 542 normal glucose tolerant (NGT) individuals in 185 nuclear families from the FUSION study. Using genotypes for (a) >300,000 SNPs from the Illumina HumanHap300 BeadChip available on 240 of these individuals, and (b) genome scan/microsatellite data on all 542 of these individuals and additional family members, we imputed genotypes on ~2.1 million common autosomal SNPs in the 542 individuals. Imputation was in two stages. First, we imputed genotypes from SNPs from the HapMap CEU sample in the 240 FUSION GWA individuals. Second, we used the microsatellite genotypes to identify regions of IBD sharing between family members to support imputation of SNPs in the non-GWA individuals. We tested for trait-SNP association between normalized residuals of S_i, DI, AIR, and S_g after adjustment for sex, age, age^2, and birthplace under an additive model. Imputation more than doubled our available sample for association testing, resulting in substantially increased power compared to analysis of the GWA sample alone. Initial analysis revealed substantially strengthened evidence for association of AIR with variants upstream from a known T2D-predisposing locus. We currently are completing the analysis of all four traits genome-wide.

An inherited balanced translocation t(2;11)(p21;p15.1) disrupting PAX6 in a patient with bilateral aniridia. *L. A. Brown[1], D. Chai[1], A. Chou[1], R. Rupps[2], C. F. Boerkoel[2], P. Eydoux[1]* 1) Children's & Women's Health Centre of British Columbia, Vancouver, British Columbia, Canada; 2) Department of Medical Genetics, Children's & Women's Health Centre of British Columbia, Vancouver, BC Canada.

Aniridia is a rare developmental defect of the anterior segment of the eye. It can occur sporadically, but is often inherited in an autosomal dominant fashion. One third of patients with isolated aniridia are found to have a chromosomal abnormality involving chromosome 11p13 or a microdeletion of the region surrounding the PAX6 gene. The risk for an association with Wilms tumor in cases with aniridia occurs in WAGR (Wilms' Tumor-Aniridia-Genitourinary Anomalies-Mental Retardation) syndrome due to a deletion that includes the WT1 gene. We report a 3-year-old boy with familial bilateral aniridia. Cytogenetic analysis of the child revealed a balanced translocation between the short arms of chromosome 2 and 11, with breakpoints at 2p21 and 11p15.1. FISH for PAX6 and WT1 revealed no disruption of either gene. Subsequent FISH using probes distal to PAX6 confirmed the breakpoint to be between 11p13 -->11p15.1. Array CGH findings revealed that the child carried a paternally inherited interstitial deletion of 280 kb within 11p13. This deletion disrupts the ELP4 gene on the telomeric end and the PAX6 gene on the centromeric end. The breakpoint involving PAX6 was proximal to the locus recognized by the RP11-133E13 FISH probe and thus no modification of the gene was observed. The inherited translocation resulting in deletion of the PAX6 gene is the likely responsible factor for the patients aniridia. There is apparently no phenotype associated with the deletion of ELP4. Cryptic deletions involving both PAX6 and ELP4 have been previously described, although the majority occurred in patients with sporadic isolated aniridia. In addition, there are several reported cases of aniridia associated with a balanced chromosome translocation and an 11p13 breakpoint. The presence of a chromosomal rearrangement in this patient demonstrates the significance of genetic counseling for this patient as his offspring may inherit the balanced translocation and the subsequent disruption of the PAX6 gene.

Identification of small molecules suppressing rCGG-repeat-mediated neurodegeneration. *A. Qurashi, L. Ray, H. Liu, P. Jin* Dept Human Molec Genetics, Emory Univ Sch Medicine, Atlanta, GA.

Fragile X-associated tremor/ataxia syndrome (FXTAS) is a progressive neurodegenerative disorder recognized in fragile X premutation carriers. Using Fruit fly, we have previously demonstrated that elongated noncoding CGG repeats in FMR1 allele as the pathogenic cause of FXTAS. Here we are utilizing this FXTAS fly model to identify small molecules that can ameliorate rCGG-mediated neuronal toxicity and lethality. We screened a collection of 2,000 FDA-approved, biologically active and structurally diverse small molecules for their ability in restoring the viability in rCGG-repeat expressing flies. We identified 20 compounds that could reverse the lethality with several of them potentially targeting to glutamate and inflammatory pathways. We further tested the effects of selected compounds on other established phenotypes of rCGG repeat expressing flies. Particularly Fluocilone Acetonide, besides rescuing lethality, can also rescue the locomotor abnormalities of rCGG-expressing flies. Fluocilone Acetonide targets and inhibits phospholipase A2 enzyme that mediates inflammation and oxidative stress. Through genetic screen, we identified a PLA2 fly ortholog that specifically modulates rCGG-repeats-mediated neuronal toxicity, but not polyglutamine-mediated toxicity. Our results demonstrate the utility of a Drosophila model for screening small molecule libraries, and suggest a specific role that PLA2 could play in the molecular pathogenesis of rCGG-mediated neurodegeneration.

Polymorphisms in the FTO gene are associated with type 2 diabetes in a population of South Asians. *L. de Koning[1], J. K. Haladyn[1], C. Xie[1], R. Do[2], A. Montpetit[3], P. Pais[4], S. Haque[5], K. Kazmi[6], T. Jayalath[7], S. Yusuf[1], J. C. Engert[2], S. S. Anand[1], Interheart genetics investigators* 1) Population Health Research Institute, McMaster University, Hamilton, Ontario; 2) Department of Human Genetics, McGill University, Montreal, Quebec, Canada; 3) The McGill University and Genome Quebec Innovation Centre, Montreal, Quebec, Canada; 4) Department of Medicine, St Johns Medical College, Bangalore, India; 5) Department of Cardiology, Bangabandhu Sheikh Medical University, Bangladesh; 6) Department of Cardiology, Aga Khan University, Karachi, Pakistan; 7) Professorial Medical Unit, Teaching Hospital, Peradeniya, Sri Lanka.

Background: Single nucleotide polymorphisms (SNPs) in the FTO gene are associated with an increased risk of type 2 diabetes (T2D), which may be mediated through increased body mass index (BMI). South Asians have an increased risk of T2D even at normal BMI. Objectives: To determine associations of SNPs in the FTO gene with T2D and HbA1c after adjusting for age, sex and adiposity measures (BMI, waist circumference[WC], hip circumference[HC] and waist to hip ratio[WHR]) in South Asians. Methods: Individuals of South Asian (n=1867) origin from the Interheart study were genotyped for FTO SNPs rs1421085, rs17817449, rs8050136, rs3751812, and rs9939609. Adiposity measures were measured in clinic, and T2D status was by self-report. Multiple regression with additive and recessive models was used for analysis. Results: Minor allele frequencies for FTO SNPs were 0.33 to 0.36. FTO SNPs were in strong linkage disequilibrium (pairwise R2 > 0.9). Under a recessive model rs17817449, rs3751812, and rs9939609 were significantly ($p<0.05$) associated with BMI but not with WC, HC or WHR. Under an additive model rs1421085, rs17817449, rs8051036, and rs3751812 were significantly associated with T2D. FTO SNPs rs1421085, rs8050136, rs3751812 and rs9939609 were significantly associated with HbA1c. Associations with T2D and HbA1c were not affected by adjustment for adiposity measures. Conclusions: In a population of South Asians, sequence variation in the FTO gene is associated with T2D, which is not mediated through adiposity measures.

Pedigree-Free Identity-By-Descent Mapping Applied To Large Scale SNP Genotyping Studies. *B. Merriman, Z. Chen, S. Strom, S. F. Nelson* Dept Human Genetics, Univ California, Los Angeles, Los Angeles, CA.

Large scale SNP genotyping studiesinvolving thousands of unrelated cases typed on whole-genome high density SNP Chipsare becoming increasingly common in genetic disease research. These are typically done in the context of linkage or association studies to localize the disease genes, but if any of the underlying risk factors has a strong founder effect, it can be more powerful to look specifically for this signature. Pedigree-Free Identity by Descent Mapping is technique we have been developing that has novel power to identify risk alleles with a strong strong founder effect, which is detected as seemingly unrelated individuals sharing a large, extended haploytpe. This method uses the high-density SNP genotyping data to directly infer large shared DNA fragments between such affected individuals, without relying on any pedigree information. We have demonstrated the basic power of the this approach in small scale studies, but if the are N subjects, the comparison process requires ~ N x N comparisons to find shared fragments, which is computationally costly when N is in the thousands. Moreover, finding fragments shared by 3 individuals requires ~ N x N x N comparisons, which becomes entirely impractical, as do all direct searches for higher order sharing. Here, we introduce an optimally efficient method for computing all large fragment sharing instances (sharing between pairs of subjects, triples, quadruples, etc) that scales like N in computational time. We illustrate this method by mapping all possible instances of apparent large IBD fragment sharing in the N ~ 18,000 Wellcome Trust Case Control samples genotyped on the Affymetrix 500k SNP set, and in the N ~ 2000 independent probands in the AGRE Autism Family samples genotyped on the Illumina 500k SNP set. We also use the ~1000 AGRE Affected Sibling Pair families to estimate the efficiency of Pedigree-Free IBD at detecting sibling IBD shared fragments, relative to rigorous IBD sharing calculations for the sibs using the parental genotypes.

Worldwide collection of genotype and phenotype data for HNPCC colon cancer and a model for the Human Variome Project (HVP). *R. G. H. Cotton[1], F. A. Macrae[2], Collaborators in the HVP and InSiGHT* 1) Genomic Disorders Research Centre, Melbourne, Australia; Human Variome Project; Department of Medicine, The University of Melbourne; 2) Department of Colorectal Medicine and Genetics, The Royal Melbourne Hospital, Parkville, Australia; InSiGHT.

InSiGHT has embraced the HVPs vision, and directed its attention to coordinating the submission of variant information derived from a wide range of diagnostic and research laboratories in Europe, Africa, USA, Canada, Japan and Australia, to its Locus Specific Database (www.insight-group.org). In parallel, integration of other mismatch repair databases serving useful functions of collating the literature, and other helpful annotations, is being unified onto a common LOVD platform to allow ready cross database interrogation. This is being supported by academic interest in defining search strategies used in clinical/laboratory services, to locate published literature relating to the variants in question, and subsequently developing automated text searching strategies to populate the InSiGHT databases. Complementing this, dedicated InSiGHT committees are formulating databases to capture phenotype data across clinical, functional and histopathological domains, again linked to variant findings. The aim is to develop a common portal to allow searching of all available data relating to particular variants, informed by generic databases, e.g. ENSEMBL, to allow the best informed interpretation of unclassified variant pathogenicity. To this end, an international, regionally represented, expert committee engaging a range of disciplines, has been assembled by InSiGHT to provide the best available interpretation of the variant data, with a process of dynamic updating. Interaction with EBI, NCBI and GEN2PHEN received a strong foundation through a meeting of the HVP in May. Mutual benefits to each have been explored, and a RoadMap for this and the entire process described. This work involving a team of committed bioinformatics teams and information system expertise, interacting with clinicians, geneticists and privacy experts, is proving a model for extrapolation to other genes and LSDBs worldwide.

Genome-wide association study of 2,400 cases of three common diseases and 1,000 shared controls in Taiwan. *C. H. CHEN, J. Y. WU, P. CHEN, M. M. T. LEE, J. Y. CHEN, S. F. HO, H. W. CHEN, C. L. HSU, C. S. J. FANN, A. T. A. CHENG, W. H. PAN, Y. T. CHEN* Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan.

BACKGROUND We carried out a joint genome-wide association study to identify susceptibility genes to common diseases among Taiwan Han-Chinese, who account for 98% of the general population residing in Taiwan. **METHODS** A total of 2,400 cases, 1000 with bipolar I disorder, 1,000 with type 2 diabetes mellitus, and 400 with young onset hypertension, were recruited through the Taiwan Multicenter Genetic Medical Project and 1000 random controls were selected from The Han Chinese Cell and Genome Bank in Taiwan. All of the 3,400 samples were genotyped using the Illumina Hap550duov3 chip and the deCode CNV chips. Individual ancestry was estimated based on sets of random selected SNPs and compared with the Asian (CHB+JPT), Caucasian (CEU), and African (YRI) samples of the HapMap project. Genome-wide association analysis was carried out with genotype and allele-type tests as well as the Armitages trend test. Models with dominate, recessive, and additive inheritance modes were also considered. In addition, multi-pointanalyses were applied to summarize regions with multiple significant points. **RESULTS** For the 3,400 samples, 537,207 out of 561,466 SNPs (95.68%) have showed polymorphism and giving an average call rate of 99.91%. All of our samples shared similar genetic background with the Asian group of the HapMap project and indicated no substantial population structure existed among the samples. For bipolar I disorder, 2 SNPs were identified with significant signals ($p < 10^{-7}$) while 11 SNPs and 3 regions showed positive evidence ($10^{-7} < p < 10^{-5}$); for young onset hypertension, 8 SNPs and 4 regions showed positive evidence; for type 2 diabetes mellitus, 16 SNPs and 4 regions showed positive evidence. Copy number variation analysis with various algorithms showed a few sites were in association with the diseases separately. **SUMMARY** We have identified novel SNPs/regions associated with bipolar I disorder, type 2 diabetes mellitus, and young onset hypertension in Han-Chinese patients.

The absence of endothelin-2 (Edn2) partially rescues photoreceptor (PR) death in inherited PR degenerations (IPDs). *A. Bramall[1], L. Pacione[1], M. Szego[1], S. Boye[2], P. D'Orleans-Juste[3], W. Hauswirth[2], M. Yanagisawa[4], R. McInnes[1]* 1) Prog in Dev Biol, Hosp for Sick Children, Toronto, ON; 2) Dept Ophthalmology, Univ of Florida, Gainesville, FL; 3) Dept Pharmacology, Univ de Sherbrooke, Sherbrooke, QC; 4) Dept Mol Genetics, Univ of Texas SW Med Center, Dallas, TX.

IPDs are the most common monogenic cause of blindness in humans; the mutant PRs are at a constant risk of death (Clarke et al. Nature 2000). Using microarrays and qPCR to identify genes that mediate the constant risk, we found that the *Edn2* mRNA was 32-fold, 70-fold and 72-fold increased (all $p<0.005$) in the *Rds*$^{+/-}$, *Tg(RHO P347S)* and *Rd1*$^{-/-}$ mouse models of IPD, respectively; *in situ* hybridization revealed increased *Edn2* mRNA only in the mutant PRs. To determine if the increased Edn2 is pathogenic, we generated PR mutant mice with Edn2 loss-of-function alleles. At age 40 and 15 days respectively, *Edn2*$^{-/-}$ *; Tg(RHO P347S)* and *Edn2-/- ;Rd1*$^{-/-}$ retinas showed a 17% ($n=6$; $p<0.003$) and 33% rescue of PR degeneration ($n=5$; $p<0.007$); by 40 days, no rescue was detectable in the slower degenerating *Edn2*$^{-/-}$ *; Rds*$^{-/-}$ retinas. Unexpectedly, *Edn2*$^{-/-}$ *;Rd1*$^{-/-}$ retinal explants did not show any PR rescue at PN17, suggesting that the rescue effect might be mediated by a secondary consequence of deleting Edn2. To further separate extraocular from ocular effects of the Edn2 deletion on PR death, we restored *Edn2* mRNA expression to *Rd1*$^{-/-}$ levels in *Edn2*$^{-/-}$ *;Rd1*$^{-/-}$ PRs by AAV-mediated gene transfer. Re-introduction of *Edn2* mRNA in *Edn2*$^{-/-}$ *;Rd1*$^{-/-}$ mice did not restore degeneration rates to that of *Rd1*$^{-/-}$ mice ($n=6$; $p>0.05$). We conclude that 1) the Edn2$^{-/-}$ rescue is one of the largest rescue effects on genetic PR degeneration yet identified; 2) the Edn2$^{-/-}$ rescue appears to be mediated extraocularly; 3) since Edn2$^{-/-}$ mice have lung disease, we examined Epo levels: Epo is 15-fold up-regulated in Edn2$^{-/-}$ mice ($n=5$) suggesting that the Edn2$^{-/-}$ rescue is due either to chronic hypoxia, increased Epo, or both. These results are consistent with a rescue of *Rds*$^{-/-}$ and light damage retinas by Epo (Rex et al Mol Ther 2004), and have therapeutic implications for human IPDs.

Highly punctuate patterns of population structure on the X chromosome. *C. A. Lambert[1], C. Connelly[1], J. Madeoy[1], R. Qiu[2], M. V. Olson[1,2], J. M. Akey[1]* 1) Department of Genome Sciences, University of Washington, Seattle, WA; 2) University of Washington Genome Center, Seattle, WA.

It is well known that overall levels of population structure are higher for X-linked markers relative to autosomal markers in humans. Because the X chromosome has a smaller effective population size relative to the autosomes, genetic drift is expected to be stronger, resulting in higher levels of population differentiation at X-linked loci. Despite this knowledge, there have been surprisingly few analyses on the spatial distribution of population structure along the X. Using publicly available data from the HapMap Project and Perlegen Sciences, Inc., we show a strikingly punctuate pattern of X chromosome population structure. Specifically, 88% (757) of HapMap SNPs with the top one percent of F_{st} values are clustered in four distinct loci. The largest of these loci spans 5.9 Mb and contains nearly 70% (595) of the most highly differentiated HapMap SNPs on the X chromosome. The clustering we observe does not appear to be an artifact of ascertainment bias present in the HapMap data, nor is it specific to the populations genotyped in the HapMap Project. Additional analyses and resequencing data suggest that these four loci have been substrates of recent, strong, adaptive evolution. Thus, natural selection as well as neutral forces like genetic drift have contributed to the differences in extant patterns of variation between autosomal and X-linked markers.

Genome scan in two large families and 33 medium sized families with childhood absence epilepsies. *M. Tanaka[1, 2], J. Bailey[2, 3], B. Minassian[4], M. E. Alonso[5], I. Martinez-Juarez[2, 5], M. Medina[6], R. Duron[2, 6], J. Machado-Salas[2], D. S. Bai[2], G. Pineda[2], I. Pascual-Castroviejo[7], A. Delgado-Escueta[2]* 1) Dept Pharmacology, UCLA, Los Angeles, CA; 2) Epilepsy Genetics/Genomics Laboratory, Neurology Service, GLAVA &. UCLA Los Angeles, CA; 3) Dept of Epidemiology, UCLA, Los Angeles, CA; 4) Genetics and Genome Biology, Hospital for Sick Children, Toronto, Canada; 5) National Institute of Neurology & Neurosurgery, Mexico City, Mexico; 6) National Autonomous University of Honduras, Tegucigalpa, Honduras; 7) Pediatric Neurology, University Hospital La Paz, Madrid, Spain.

We recently discovered three GABRB3 mutations on chromosome 15q 11-12 in 4 families (8%) out of 48 families with remitting Childhood absence epilepsy (CAE). (2008 Tanaka et al). The aim of the present study is to identify other novel loci for absence epilepsy. Genome scan with 440 microsatellites was performed in (a) 33 medium sized families with 400 members (b) 2 large families, namely, family M-17 (four generations with 58 members of which 9 were clinically and 3 EEG affected) and family LA-40 (four generations with 58 members of which 11 were clinically and 1 EEG affected). Each family was ascertained through a proband with CAE . easy LINKAGE program was used for mathematical analyses using 2 diagnostic models assuming 70% penetrance , disease allele frequency of 0.001 and phenocopy and gene mutation rates of 1%. In family M-17, the highest LOD scores were 3.3 (theta=0m=f) for chromosome 4q13 (D4S409) and 2.08 for chromosome 4q35 (D4S1535). However, haplotypes built around D4S409 and around D4S1535 did not segregate with affecteds in family M-17. Because pooled LOD scores in the 33 medium sized families was 3.3 for D12S1045 in chr.12q24.3 and 2.7 for the same D12S1045 in chr.12q24.3 for family M-17 and because the highest LOD score was 2.7 (theta=0m=f) for chr. 10q11 (D10S1220 and D10S1239) in family LA-40, we are looking for recombinations and building haplotypes around chr.12q24.3 and 10q11 in families M-17 and LA-40 and the 33 medium sized families. Present results indicate locus heterogeneity in CAE and we hope to identify the absence causing genes in chr. 12q24.3 and 10q11.

Identification and validation of novel regions associated with sporadic amyotrophic lateral sclerosis (ALS). *J. Huang*[1], J. Jaworski*[2], Y. Yang[1], W. Chen[1], N. Siddique[1], E. Rampersaud[2], H. Khan[1], E. Liu[1], M. Zheng[1], J. Armstrong[1], R. Sufit[1], S. Heller[1], J. L. Haines[3], M. A. Pericak-Vance[2], T. Siddique[1]* 1) Department of Neurology,Northwestern University; 2) Miami Institute for Human Genomics, Miller School of Medicine, University of Miami; 3) Center for Human Genetics Research, Vanderbilt University.

Sporadic ALS (SALS) is a severe neurodegenerative disorder affecting both the upper and lower motor neurons. The genetic etiology of SALS is unknown but believed to be caused by both genetic and environmental factors. To identify genetic variants that predispose to SALS, we performed a case-control genome-wide association study (GWAS) in a US Caucasian sample. We genotyped 1,045 samples (524 cases and 521 controls) using the Illumina 319K genotyping platform. Following quality control checks (assuming a HWE threshold p>0.0001, MAF threshold> 0.10, sample call rate> 98.5%, and SNP efficiency > 95%), 970 samples (488 cases and 482 controls) and 274,494 SNPs remained for analysis. The mean age at onset for cases (and at ascertainment for controls) was 49 12 yrs. Association analyses were performed using the Armitage Trend test in PLINK. We validated our results using publicly available genotype data for 536 samples from the National Institute of Neurological Disorders and Stroke (NINDS) repository. We identified 2,795 SNPS with a P < 0.01, more than would be expected by chance. Our top 10 hits were observed on chromosomes 16, 10, 11, 3, and 7 with the most significant marker on chromosome16 (rs12446182, P = 7.89 x 10-7). To validate our findings we identified SNPs associated with SALS (P<0.05) in either our GWAS or the independent GWAS performed in the NINDS dataset. We then performed a combined meta-analysis of the 708 analyzable SNPs that overlapped both sets. This analysis revealed one SNP (rs130110) on chromosome 22 that met a Bonferroni-corrected significance threshold (P = 3.66 x 10-7, OR=1.84) in the combined dataset of 1,507 samples from both GWAS. Rs130110 lies in intron 1 of FAM19A5, a gene whose protein is expressed primarily in the brain. These results strongly support the the role for multiple genes of modest effect in SALS.

The contribution of Aneusomy 15q25qter to the Aetiology of the Shprintzen-Goldberg syndrome. *D. Tegay[1], P. Papenhausen[2], J. Tepperberg[2], O. Nahum[3], B. Pletcher[4], A. Shanske[5], B. Levy[3]* 1) Med & Med Gen, NY Col Osteopathic Med at NYIT, Old Westbury, NY; 2) LabCorp Inc. Cytogenetics, RTP, NC; 3) Columbia University, Dept of Pathology, New York, NY; 4) Newark, NJ; 5) Children's Hosp Montefiore, Bronx, NY.

The hallmark of Shprintzen-Goldberg syndrome (SGS) is craniosynostosis & marfanoid habitus. The etiology of SGS is uncertain & a single case report links SGS to a mutation in the fibrillin-1 gene (15q21.1). Tetrasomy 15q in the form of a neocentric marker chromosome (NMC) is a rare disorder with only a handful of reported cases, the majority of which have been mosaic. We have identified 3 patients with a non-mosaic NMC resulting in tetrasomy of the distal region of 15q. CENP proteins, FISH, CGH & microarray analysis were used to characterize the NMCs. Case 1: 37 wk gestation, BW of 2317g & length 47cm. Horseshoe kidney with hydronephrosis, metopic & bilateral coronal craniosynostosis that required craniotomy at 5mnth. Non-verbal at 4½ yrs with Turricephaly, thoracodorsal scoliosis, bilateral contractures of digits 2, 3 & 4. Case 2: Evaluated at 32yrs for overgrowth, marfanoid habitus & mild MR. His BW was 5900g & length 63cm. Surgery for craniosynostosis at 8 mnths & scoliosis at 29yrs. At 32 he was 193.5 cm tall with an HC of 57cm. He had dolicocephaly, malar flattening, low-set ears, a pseudo-cleft palate, retrognathia, pectus deformity & was globally delayed. Case 3: FT pregnancy, BW of 3239g. GR at 6yrs with macrocephaly, turricephaly, & very high vaulted & narrow palate with a bifid uvula, kyphoscoliosis, long extremities, flexion contractures of the digits & was non-verbal. Microarray analysis showed the breakpoints in cases 1-3 to be 15q25.2-qter (17.3Mb), 15q26.1-q26.3 (11.3Mb) and 15q26.1-qter (16Mb) respectively. FISH confirmed the tetrasomic nature of the NMC. The striking phenotypic resemblance in our 3 cases points to a role of genes in distal 15q in the etiology of SGS. The 15q24-26 region is rich in segmental duplications which may promote rearrangements of this area. Future studies will attempt to assess how gene dosage or dysregualtion of genes in 15q25-qter contribute to the SGS phenotype.

Mutational screening of regulatory regions flanking GBA in patients with Gaucher disease. *Y. Blech-Hermoni, E. Barnoy, S. Ziegler, B. Stubblefield, K. Hruska, M. LaMarca, N. Tayebi, E. Sidransky* Medical Genetics Br, NHGRI/NIH, Bethesda, MD 20814.

BACKGROUND: Gaucher disease (GD) is the most common lysosomal storage disorder. Almost 300 mutations have been identified in the gene encoding glucocerebrosidase (GBA), the enzyme deficient in GD, resulting in the accumulation of uncleaved substrate in cells of the reticuloendothelial system. Genotype-phenotype correlation in GD remains limited, encouraging the identification of other modifiers that could underlie the high variability in residual enzyme activity and disease severity in patients with the same genotype. The regulatory architecture of GBA has been investigated previously, identifying functional cis-acting regulatory elements within the proximal promoter region of the human gene locus (Doll et al., 1995; Moran et al., 1997), and c-Myc binding sites within 5kb of the translation initiation site. METHODS: We used computational multispecies comparative approaches to identify evolutionarily conserved sequences in the GBA gene locus. Site-directed mutatgenesis was performed to generate constructs with alterations at these specific sites. We used the dual-luciferase expression assay to validate the functional role of these binding sites in COS-7 cells. Finally, we sequenced predicted and previously reported regulatory sites in patients sharing the same genotypes. RESULTS: Our computational analysis of the locus identified two clusters of predicted transcription factor binding sites. Expression assays revealed that changes in six predicted sequences resulted in dramatic downregulation, whereas changes in two sites resulted in statistically significant increases in reporter gene expression. Each sequence is being examined in patient samples to determine whether alterations at these sites impact phenotype. CONCLUSIONS: Computational multispecies comparative approaches identified potential GBA regulatory regions that appear to impact gene expression, providing sites for further evaluation in phenotypically diverse patients with GD.

Promoter variants of endothelial lipase (*LIPG*) are modulators of *LIPG* gene expression and HDL-C concentration in humans. *S. Khetarpal[1], A. Edmondson[1], H. Neeli[2], S. Kathiresan[3], L. A. Cupples[4], S. Demissie[4], A. Manning[4], S. DerOhannessian[1], M. Wolfe[1], D. Rader[1]* 1) Institute for Translational Medicine and Therapeutics, University of Pennsylvania School of Medicine, Philadelphia, PA; 2) Temple University, Department of Hospital Medicine, Philadelphia, PA; 3) Broad Institute of Harvard and MIT, Cambridge, MA; 4) Boston University and Framingham Heart Study.

Increased blood levels of HDL cholesterol (HDL-C) are associated with decreased risk of developing cardiovascular disease. While few genes have yet been implicated in directly increasing HDL-C concentration, one potential candidate is endothelial lipase (*LIPG*), expression of which in animal models is inversely associated with HDL-C levels. We resequenced the *LIPG* promoter in human subjects from extremes of the HDL phenotypic distribution. We identified an excess of rare promoter variants unique to subjects with elevated HDL-C. We also identified a common promoter haplotype. We hypothesized that promoter variants may alter *LIPG* expression and thus alter HDL-C metabolism. To test this hypothesis, we generated a firefly luciferase reporter construct driven by the *LIPG* promoter. Regulatory variants identified through resequencing were introduced into the luciferase reporter construct via site-directed mutagenesis. Many of the variants identified specifically in high HDL-C subjects were found to significantly decrease gene expression in vitro, consistent with reduced *LIPG* expression leading to elevated HDL-C. Genetic association analysis of the common promoter haplotype in the Framingham Heart Study showed that the common *LIPG* promoter haplotype is significantly associated with decreased HDL-C. The luciferase reporter construct containing the promoter haplotype exhibited increased expression in vitro, as predicted. Subjects with the common *LIPG* promoter haplotype were also found to have significantly increased plasma levels of EL protein, as determined by ELISA. Our results suggest that EL is an important modulator of HDL-C metabolism in humans, with regulatory variation of *LIPG* playing an important role in modulating HDL-C concentration.

The Impact of Small Sample Size on the Robustness of Exploratory Pharmacogenetic Studies. *A. H. Beecham[1], M. R. Nelson[2], G. G. Koch[1]* 1) Biostatistics, University of North Carolina, Chapel Hill, NC; 2) GlaxoSmithKline, Research Triangle Park, NC.

The potential for pharmacogenetics to have an impact on drug development is greatest if its contribution to safety or efficacy can be identified in early phase studies. This requires that statistical analyses of genetic studies be conducted with small sample sizes. However, there have been few efforts to characterize the robustness of statistical methods used for such small samples in early phase trials. To quantify robustness, metrics were used from both quantitative trait (QT) and case-control (CC) endpoints related to idiosyncratic hepatotoxicity on genetic and clinical data from early phase development programs. Sixty genetic markers were analyzed in 195 subjects for the QT analysis and 11 cases and 155 controls in the CC analysis. For the QT analysis, wherein the endpoint was strongly skewed, diagnostics were calculated to test the validity of the homogeneity of variance and normality of residuals assumptions. Violations in these assumptions were found so deviance corrected statistics, GEE based empirical Wald and Score statistics, a gamma model, and proportional hazards regression were investigated to address the violations. For each method the effective type I error was computed using a permutation procedure. For the CC analysis the effective type I errors were calculated for the mid-p, Fishers exact, Pearson chi-square, exact Pearson chi-square, likelihood ratio chi-square, exact likelihood ratio chi-square, and Wald chi-square. In the presence of small sample sizes, the normal and gamma model-based and deviance corrected statistics as well as proportional hazards regression tend to overstate association, while the empirical Wald statistic vastly overstates association and empirical score tends to understate association. For all markers the gamma is a better fit than the normal. For the CC analysis, the mid-p operates at the nominal level, while exact tests tend to understate and approximate tests overstate association. These results provide some guidance on selecting appropriate testing procedures for exploratory pharmacogenetics analyses in small sample sizes.

Introduction of prkar1a -/- Mouse Embryonic Fibroblasts in Nude Mice Leads to Tumor Formation: Gene expression after recombinant mouse growth hormone treatment. *S. Boikos, M. Moschovi, M. Nesterova, E. Bimpaki, K. Tsang, C. Cheadle, T. Watkins, YC. Chen, M. Abu-Asab, AF. Parlow, G. Chrousos, CA. Stratakis* SEGEN DEB NICHD, NIH, Bethesda, MD.

Human growth hormone (GH) therapy in cancer patients is controversial because of its potential effects on tumor growth. Patients with Carney complex and PRKAR1A mutations develop acromegaly and various tumors. We wanted to study the effects of recombinant mouse GH (rmGH) on growth of a tumor grown in nude mice after injection of prkar1a-/- mouse embryonic fibroblasts (MEFs). 20 male and 20 female mice (6 weeks and 6 months old), were used for the experiment: all received injections of immortalized $2x10^6$ prkar1a-/- MEFs after soft agar selection. ; all mice formed tumors. Mice received also either placebo or 50 microg/twice-a-day of rmGH. In the 6 weeks age group, male mice treated with rmGH had greater tumor size (24.51 vs. 19.75 1.71 mm, $p<0.01$) than those that were not treated with rmGH. There were no other statistically significant differences. In order to identify genes regulated by Prkar1a and growth hormone, gene expression was assessed by oligonucleotide microoarrays using the Illumina MouseRef8 V2 Expression BeadChip for microarrays. Z-transformation for normalization was performed on each Illumina sample/array. Using a cut-off of >/=2 times,60 genes were upregulated and 26 were downregulated in the tumors grown in male nude mice with GH injection vs male nude mice w/o GH injection . Most of the upregulated genes (Ecm1, s100a8, s100a9 , Esm1, TM4SF3, Itgr1, Dusp6, KLRA4, KLRA15, KLRA16, KLRA18, KLRA20, KLRA33, SOCS2) have been found to be cancer-related. Mouse Klra receptors inhibit natural killer cell cytotoxic activity which are thought to mediate anti-tumoral immunity. SOCS2 suppresses the cytokine signalling and regulates growth hormone signaling. We conclude that rmGH increased the growth of tumors formed by prkar1a-/- MEFs in male nude mice at a young age. These data suggest an age and gender-specific effect of excess GH in prkar1a-induced tumorigenesis. Ongoing studies aim at further investigating the interaction of these genes with the PKA signaling and related pathways.

Polymorphism associated with ORMDL3 expression Defines Non-Atopic Early Childhood Asthma. *H. Bisgaard[1], P. M. A. Sleiman[3], K. Bonnelykke[1], M. Brasholt[1], B. Chaves[1], E. Kreiner-Moller[1], M. Stage[1], C. E. Kim[1], S. F. A. Grant[1], R. Tavendale[2], C. B. Piper[1], C. N. A. Palmer[2], H. Hakonarson[3]* 1) Copenhagen Studies on Asthma in Childhood; University of Copenhagen; 2) Population Pharmacogenetics Group, University of Dundee; 3) Center for Applied Genomics, The Childrens Hospital of Philadelphia, University of Pennsylvania School of Medicine.

A recent GWA identified an asthma predisposition locus on 17q12-q21. Here, we replicate the reported association in the Danish population and further investigate association between a tagging variant and the development of asthma associated phenotypes, including recurrent wheeze, asthma, acute severe exacerbations, eczema, rhinitis and allergic sensitization as well as lung function and bronchial responsiveness (BH) assessed longitudinally from neonatal age to school age. Case control analysis of the COPSAC mothers identified significant association between rs7216389 and asthma (P=3x10-4). TDT confirmed over-transmission of the risk T allele to the affected offspring. Homozygosity of the risk T allele was significantly associated with the development of recurrent wheeze, asthma and acute severe exacerbations (hazard-ratio 2.15 [1.43-3.21], p=0.0002). Children with the TT-genotype had an increased risk of severe exacerbations that persisted from 1 to 6 years of age (incidence ratio 2.48 [1.42-4.32], p-value=0.001) and a corresponding increase in objective assessment of BH from early infancy to school age. Variation at the chromosome 17q12-q21 locus, tagged in this study by the rs7216389 polymorphism, is associated with approximately 2-fold increased risk of asthma, asthma exacerbations and BH from early infancy to school age. The effect size suggests that this locus is the most important currently identified genetic determinant of preschool asthma. Variation at this locus is associated with specific asthma sub-phenotypes characterized by early onset, persistent risk of severe exacerbations, and bronchial hyperresponsiveness but without conferring risk of eczema, rhinitis or allergic sensitization. As such, this locus is the first to harbour variations that defines a particular non-atopic asthmatic phenotype in early childhood.

Diabetes Genetic Variation Pathway: one step toward the personalized medicine. *B. Oh[1], J. Lim[1], J.-T. Woo[2], H. Park[1]* 1) Medical Engineering, School of Medicine, Kyung Hee University, Seoul, Korea; 2) Endocrinology and Metabolism, School of Medicine, Kyung Hee University, Seoul, Korea.

The prevalence of diabetes mellitus has been epidemic worldwide with the greatest increase in Asia, Africa, and South America. The life-threatening complications of diabetes sometimes devastate patients from the retinopathy, nephropathy, lower-limb amputation caused by the continued exposure of tissues to the high glucose. Since the hyperglycemia could be prevented and reversed greatly by changing the life style including the exercise and nutrition, the diabetes risk tests, such as one provided by American Diabetes Association, have been used to alarm the high risk group. However, the increase of diabetes incidence has not been stopped for the last decade, demanding new approaches. Following the growth of genomics, the disease susceptibility of human genetic variations has successfully been explored to provide the better understanding for the development of diabetes mellitus. Since the discovery of TCF7L2 gene in 2006, more than 15 genes have been confirmed to be replicated from diverse populations and the information grows to contribute their genetic variations as risk factors in the development of diabetes. In order to apply the recent discoveries about genetic risk factors in diabetes mellitus into the personalized medicine, the integration of the genetic variation information into the genetic pathway of diabetes, which so called genetic variation pathway, has been attempted. The diabetes genetic variation pathway will be utilized to provide health care providers with the different susceptibility of individuals in diabetes and will be expanded for the selection of high risk group.

Confirmatory Testing Outcomes Following Newborn Screening for Biotinidase Deficiency. *K. Cusmano-Ozog[1], F. Lorey[2], N. N. Kazerouni[2], M. Roberson[2], T. M. Cowan[3]* 1) Dept Medical Genetics, Stanford Univ, Stanford, CA; 2) Genetic Disease Screening Program, CA Dept of Public Health, Richmond, CA; 3) Dept Pathology, Stanford Univ, Stanford, CA.

Biotinidase deficiency (BD), an autosomal recessive disorder of biotin metabolism, is associated with metabolic acidosis, seizures and other serious sequelae. Symptoms are easily treated with biotin, and BD is included in many newborn screening (NBS) programs via colorimetric testing of biotinidase activity on dried blood spots. Positive screening results are followed up by diagnostic enzyme testing in serum or plasma. Such testing identifies affected individuals with absent or near-absent enzyme activity, and may also reveal partial variants and carriers of BD. Because mishandled samples are prone to enzymatic degradation and artifactually low activity, parental samples are requested to be sent as controls where possible. Since NBS for BD was introduced in California in July 2007, 464,073 newborns have been screened. This report summarizes our experience with confirmatory testing of positive results. Prior to formal implementation of BD screening, testing of newborns and parents during the pilot phase allowed for the establishment of reference ranges for normal activity (nmol/ml/min, meanSD; newborns: 4.40.5; adults: 5.20.7), as well as carriers (newborns: 2.30.2; adults: 3.10.5), partial variants (1.40.2) and profound BD (0.40.2). Following implementation, 21 newborns had positive screens; follow-up testing revealed 10 consistent with profound BD, 5 partial variants, 2 carriers and 2 with normal activity. Two patients had biotinidase activity suggestive of either profound or variant BD, but despite parental testing a definitive diagnostic category could not be assigned. Molecular studies are needed to clarify these cases. Two additional newborns had low biotinidase activity which, upon testing of parents, was shown to be due to a sample handling artifact. Our findings reveal an incidence of BD among California newborns of approximately 1/46,000, and indicate that the screening cutoff (6 ERU or 6-10 on two screens) yields a positive predictive value for profound and variant BD of 81%.

A new strategy using sapropterin and placebo to determine the predictive value of mutation analysis towards identifying BH4 responsive hyperphenylalanemia. *J. R. Utz, K. Bentler, D. Markowitz, C. Pham Lorentz, D. Erickson, B. Diethelm-Okita, C. B. Whitley* Pediatrics, University of Minnesota, 420 Delaware St SE, Minneapolis, MN 55455.

Background: Sapropterin dihydrochloride (SAP), a tetrahydrobiopterin (BH4) analogue, is FDA approved for treatment of BH4 responsive Phenylketonuria (PKU) and is the 1st prescription drug therapy approved for PKU disease. SAP responsive patients typically realize a >20% reduction in serum phenylalanine (PHE), which combined with a low PHE diet minimizes the risk of development/progression of severe neurological damage from hyperphenylalanemia (HPA). SAP responsiveness is reported in 20%-56% of patients and is indicated for indefinite, costly SAP therapy. The cost and chronic nature of SAP therapy make accurate and timely verification of responders/non-responders of utmost importance. The ability to predict the likelihood of SAP response would be advantageous towards justifying trials of SAP in HPA patients. To date, no standard methods exist for predicting SAP response. Mutation analysis (MA) has been considered for this purpose, but usefulness towards this end hasn't been established. Our institution was 1st in North America to offer MA to HPA patients and the mutations of most of our patients are known. **Objective:** We describe "START", a clinical diagnostic test designed to evaluate BH4 response; cumulative results may reveal the predictive value of MA towards determining likelihood of BH4 response. **Methods:** HPA patients are randomized in double blind fashion to SAP 20 mg/kg/day or placebo and alternate SAP with placebo weekly. Weekly PHE levels and food diaries are obtained. Weekly treatments are unblinded after 4 weeks and compared to dietary PHE intake and PHE levels. BH4 response is thereby determined and compared to MA. Finally, correlations between MA and BH4 response are cited. **Conclusion:** The determination of the predictive value of MA towards realizing the likelihood of BH4 response will decide if studies are warranted that further explore specific mutation associations with BH4 response, extent of response, and impact on clinical quality and cost-effectiveness of PKU disease management.

GATA4 Gene Deletions Identified by Clinical aCGH Analysis in Cases with Congenital Heart Defects Observed on Fetal Ultrasound. *S.-H. L. Kang[1], O. Shchelochkov[1], A. N. Pursley[1], A. M. Breman[1], C. Sheppard[2], C. A. Bacino[1], A. Dagli[3], R. T. Zori[3], S. R. Lalani[1], A. Patel[1], S. W. Cheung[1]* 1) Dept of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Hill Country Maternal Fetal Medicine, Austin, TX; 3) Dept of Pediatrics-Genetics, University of Florida, Gainesville, FL.

Deletions of chromosome 8p23 have been previously identified and characterized. The sizes are reported to range from large terminal >12Mb deletions to <2Mb interstitial deletions. Recurrent deletions have been observed that are mediated by low copy repeats, with a larger ~6Mb deletion usually detectable by routine chromosome analysis and a smaller ~3Mb deletion requiring high-resolution chromosome analysis. The *GATA4* gene maps to chromosome 8p23.1 and is known to cause congenital heart defects when haploinsufficient. The larger recurrent deletion includes the *GATA4* gene, but smaller deletions sometimes may not. DNA microarrays used for clinical diagnosis are usually enriched for chromosome regions that contain dosage sensitive genes. Although the referral for aCGH testing is now commonplace postnatally when genomic disorders are suspected, it is not often considered in the prenatal period when ultrasound abnormalities are detected. Here, we present four cases where cardiac abnormalities were observed on prenatal ultrasound and the clinical aCGH test detected a recurrent deletion of at least 2.9Mb in size on chromosome 8p23.1 including the *GATA4* gene. Two of these cases were diagnosed prenatally (twins; case 3 and 4) and two in the neonatal period (case 1 and 2). Chromosome analyses were also performed prior to or concurrently with aCGH on all four cases and an interstitial deletion of chromosome 8p23.1 was detected in three of the four cases (1, 3, and 4). Importantly, involvement of the *GATA4* gene is not distinguishable by karyotype analysis alone. In addition, case 2 was also evaluated for possible DiGeorge syndrome by FISH analysis before the aCGH test was ordered. These cases further demonstrate the superior value of aCGH analysis in the prenatal period, particularly when ultrasound abnormalities such as heart defects are observed.

Deep Sequencing of Two Neighboring Autism Candidate Genes: Myo1D and TMEM98. *S. Strom, B. Merriman, S. F. Nelson* Department of Human Genetics, University of California, Los Angeles, Los Angeles, CA.

Linkage analysis has identified chromosome 17q as a region linked to male-specific autism. This linkage signal is derived from a set of 69 affected brothers sharing both alleles (Z2) across this region. Association analysis highlighted several candidates, in this interval, including a locus spanning two known genes Myo1D and TMEM98. To determine if coding variants in these genes are associated with autism, all coding exons in both genes were sequenced one brother from each of the 69 male sib-ships exhibiting Z2 sharing. In total, over 50kb of sequence was generated for each individual, with an average 20x overall coverage. These data are sufficient to rule out the possibility that coding variants in these two genes contribute to autism etiology. However, sufficient coverage of intronic sequence prevents these genes from being eliminated as candidate genes.

Patterns of Genetic Variation at *ICAM-1* in Diverse African Populations. *F. Gomez[1,2], G. Tomas[3], J. Rocha[3], S. A. Tishkoff[1]* 1) The Department of Genetics, The University of Pennsylvania School of Medicine, Philadelphia, PA; 2) The George Washington University, Washington, DC; 3) Instituto de Patologia e Imunologia Molecular da Universidade do Porto, Portugal.

Malaria, resulting from infection by the *Plasmodium falciparum* parasite, causes millions of deaths each year and is one of the strongest selective forces in recent human evolution. ICAM-1 (intercellular adhesion molecule-1) is a major vascular endothelial receptor for *P. falciparum* parasitized erythrocytes, and therefore may affect susceptibility to malaria. Currently, the clinical consequences of nucleotide diversity at *ICAM-1* are not well understood. This study seeks to examine nucleotide variation at *ICAM-1* in diverse African populations to better understand the scope of genetic variation at this locus and to identify variants that may influence susceptibility to malaria. A panel of ~200 individuals originating from Nigeria, Cameroon, Ghana, Tanzania, Kenya, and the Sudan were re-sequenced across a ~7 kb region of *ICAM-1*. Individuals from Thailand and Portugal were also re-sequenced as comparative data. Using these data we have characterized levels of diversity and have used tests of neutrality to identify signatures of natural selection. Initial haplotype analyses suggest that there are more unique haplotypes in Africa than in Europe or Asia. Additionally, a well-studied SNP that may influence susceptibility to severe malaria, called *ICAM-1*$^{\text{Kilifi}}$, was identified at high frequency within Africa and Asia. In Asia *ICAM-1*$^{\text{Kilifi}}$ was identified on a single haplotype background, and in Africa this variant was found on several distinct haplotype backgrounds suggesting that it may have originated in Africa, rather than in Asia.

Prediction of SNPs affecting transcriptional regulation at complex trait susceptibility loci. *K. J. Gaulton[1], C. J. Willer[2], K. L. Mohlke[1]* 1) Dept of Genetics, University of North Carolina, Chapel Hill, NC; 2) Dept of Biostatistics, University of Michigan, Ann Arbor, MI.

Genome-wide association (GWA) studies have successfully identified loci harboring risk variants for numerous traits and common diseases. However, many of these loci have tens to hundreds of associated variants and thus identifying the variant(s) functionally responsible for an association signal often requires prioritizing between SNPs. As many functional variants likely have a regulatory effect on transcription, we developed a naïve Bayesian classifier to predict SNPs in likely regulatory elements. This approach uses a training set to derive the likelihoods that multiple conditionally independent datasets will correctly predict regulatory elements, and uses the joint likelihoods to classify SNPs. We integrated the 12.2 million records in dbSNP with 19 genome-wide datasets characterizing transcriptional regulation, such as DNase hypersensitivity, histone modifications, and transcription factor binding. After training the algorithm with SNPs in genomic regions previously shown to have positive or negative evidence of a regulatory effect, preliminary results suggest that DNase hypersensitivity and H3K4me1 are among the strongest predictors. We tested the classifier on 1,590 SNPs at 60 independent loci associated with type 2 diabetes, body-mass index, triglyceride level, HDL-C level or LDL-C level and without an obvious non-synonymous variant. 188 (11.8%) SNPs were predicted to be located in regulatory elements, an average of 3.1 per locus. Compared to all SNPs in dbSNP, more SNPs than expected by chance were located in TRANSFAC conserved transcription factor binding sites (obs=8, exp=1.6, p=4.2x10-5) and in genomic regions highly conserved across 28 species (obs=11, exp=4.5, p=5.6x10-3). The quality of predictions will continue to improve as additional independent datasets are incorporated. These results suggest that our approach may enrich for SNPs affecting regulation, providing useful information for functional studies at complex trait associated loci.

In Silico fine mapping: The example of gene expression traits in Mouse Liver. *M. A. Rivas[1,2,3], C. Cotsapas[2,3], A. Kirby[2,3], B. M. Neale[2,3], P. Little[4], T. Wiltshire[5], M. J. Daly[2,3]* 1) Dept Mathematics, Massachusetts Inst Technology, Cambridge, MA. USA; 2) Broad Institute of MIT and Harvard, Cambridge, MA. USA; 3) MGH Center for Human Genetic Research Boston, MA. USA; 4) School of Biotechnology and Biomolecular Sciences, University of New South Wales, Randwick, NSW 2052, Australia; 5) Genomics Institute of the Novartis Research Foundation, San Diego, CA. USA.

The genetics of gene expression has been proposed as a way to understand the molecular basis of phenotypes. In this study we demonstrate the power of the Mouse HapMap to perform in silico fine-mapping of eQTLs in mouse liver. We take advantage of the Mouse HapMap, a genome-wide map composed of ~130k SNP variants typed in 94 common inbred lab strains, to enable genotype-phenotype expression correlation analysis. We integrate genotype and phenotype data from 31 BXD strains and 28 classical inbred strains and combine eQTL analysis from these two studies to fine-map loci found in the BXD strains by looking at the added genetic variation captured and shorter LD segments in the classical inbred strain simultaneously. We have implemented an analytic design that addresses biases due to small sample size, over-dispersion effects of statistics, and population substructure. We extend this strategy for analyzing gene expression data to unravel how cis-acting genetic factors influencing gene expression vary from tissue to tissue. Our combined analysis shows at least 25% concordance reached by comparing eQTLs across classical inbred and recombinant inbred strains. We are able to fine-map loci found in the BXD strains by looking at the additional genetic variation and shorter LD segments in the classical inbred strain data. We can resolve eQTL intervals from 10-20 Mb down to 1-5 Mb, and in some cases much smaller intervals. This greatly reduces the burden of subsequent fine mapping to identify the causal variants, and we anticipate that this combined strategy will be applicable for many phenotypes.

Heritability of HCFC1 expression levels from the inactive X chromosome. *J. Stahl, L. Carrel* Penn State College of Medicine, Hershey, PA.

X chromosome inactivation silences one X chromosome in females to equalize X gene dosage between males and females. However, 15% escape X inactivation and are expressed from both the inactive X (Xi) and active X (Xa). An additional 10% are variable and escape in only a subset of Xs tested. How variable Xi expression contributes to normal trait variation amongst females is unknown but of utmost interest. To better understand factors influencing Xi expression, we sought to determine whether variable Xi expression patterns are heritable. We tested Xi expression in clonal lymphoblast lines from three-generation CEPH pedigrees. Using a quantitative allele-specific expression assay, we tested 14 independent Xs and identified five loci with variable Xi expression. We focused on two genes in Xq28, HCFC1 and ARD1A. Xi levels for HCFC1 in nine independent Xs ranged from 0-23% of Xa levels, while ARD1A in five Xs ranged from 0-33% of Xa levels. To determine whether Xi expression levels reflect escape in a subset of cells or low expression in all cells, we tested HCFC1 in 9 independent subclones from one individual. Two distinct patterns emerged; the five subclones carrying an inactive HCFC1 C-allele had no Xi expression, whereas four lines with an Xi T-allele had Xi levels at ~10% of Xa levels. These data suggest Xi levels are not stochastic and reflect reduced Xi expression in all cells. Further, Xi expression differences in clones from a single individual are inconsistent with autosomal inheritance for this locus. To further evaluate Xi heritability, we tested HCFC1 in informative females from one pedigree. Different Xi expression levels were seen for different Xs. One Xi tested in four individuals was 0-4% of Xa levels, whereas another tested in three individuals was 10-22% ($p<0.05$). Xi expression levels for HCFC1 appear heritable and X-linked. However, a maternal Xi allele of ARD1A significantly differed in sisters (0% and 26%), inconsistent with X-linked inheritance. These data illustrate complexity in Xi regulation and suggest both cis and trans factors impact expression. Further, the identification of epigenetic and genetic factors influencing variable escape from Xi will aid in evaluating phenotypic consequences of Xi expression.

The Fanconi anemia pathway plays a critical role in regulating telomeric recombination in ALT-immortalized human cells. *M. Meyn[1,2,3], C. Yeh[1,2], F. Al Murshedi[2,3], H. Root[1,2]* 1) Genetics & Genomic Biology, Hosp Sick Children, Toronto, ON; 2) Molecular Genetics, Univ Toronto, Toronto, ON; 3) Paediatrics, Univ Toronto, Toronto, ON.

To understand how Fanconi anemia (FA) proteins may function in genetic recombination, we are studying the role FA proteins play in the recombination-dependent ALT telomere maintenance pathway. We have found that FANCD2 localizes to telomeric foci in ALT cells, where it interacts with the telomeric protein TRF2 and the BLM helicase. We now report FA pathway proteins upstream (FANCA, FANCG and FANCM) and downstream (BRCA1, FANCJ, and BRCA2/FANCD1) of FANCD2 mono-ubiquitination form nuclear foci that localize to telomeric foci and PML bodies in ALT, but not in telomerase-positive or primary human cells. These FA-related proteins typically associate with telomeric foci containing FANCD2. Depletion of FANCD2 in ALT cells leads to telomeric entanglements, telomere dysfunction-induced foci, rereplicated DNA, mitotic failure and cell death. We now report ALT cells depleted of TRF2 show a similar phenotype. Notably, in telomerase positive cells, TRF2 depletion also results in telomere fusions followed by cell death/senescence, while telomere abnormalities are minimal after FANCD2 depletion.

Our results suggest disruption of the FA pathway causes mitotic catastrophe in ALT cells through effects on telomere homeostasis. Loss of TRF2 uncaps telomeres, leading to their recognition and processing by DNA repair proteins. We find depletion of FANCD2, but not TRF2, causes ALT-specific amplification of telomeric DNA and the appearance of nuclear filaments of the recombination protein RAD51, suggesting that FANCD2 depletion in ALT cells does not simply result in telomere uncapping. Rather, we hypothesize the FA pathway limits telomeric recombination and/or controls resolution of telomeric recombinational events in ALT cells. In the absence of a functional FA pathway, we propose that unregulated recombination causes telomere amplification and entanglement. This leads to continued cell growth without proper segregation of DNA, resulting in multiple secondary abnormalities and mitotic catastrophe.

Mechanistic insights into ring chromosome formation using SNP arrays. *L. K. Conlin[1], B. D. Thiel[1], S. A. Hosain[2], P. S. Munoz[1], J. T. Glessner[1], H. Hakonarson[1], I. D. Krantz[1], N. B. Spinner[1]* 1) The Children's Hospital of Philadelphia, Philadelphia, PA; 2) UMDNJ Robert Wood Johnson Medical School, New Brunswick, NJ.

Ring chromosomes are associated with varied clinical features depending on the chromosome and the specific sequences involved. We are particularly interested in the ring chromosome 20 (r(20)) syndrome, which is associated with a characteristic form of epilepsy. Several mechanisms for how ring chromosomes impact phenotype have been proposed including deletions at the fusion points, mitotic instability, and gene silencing due to altered chromatin conformation. We studied 16 patients with ring chromosomes to understand their composition and mechanism of formation. We analyzed 9 r(20), 3 r(X), and one each r(3), r(17), r(22), and r(Y) chromosomes by cytogenetics, FISH, and SNP array analysis. SNP array analysis provided both genomic content and inheritance information for the 47,+(r) patients, with two of the three rings showing complex meiotic inheritance. Among the 13 patients with a 46,(r) karyotype, two distinct groups were observed. First, are rings that are present as mosaics with no evidence for genomic loss. All six of these rings were present in conjunction with a normal cell line, consistent with their formation postzygotically. Second, are those that have no evidence for a normal cell line and have loss of material from the ring chromosome. All seven patients with non-mosaic rings had loss of genomic material at one or both telomeres. We propose that these rings occurred during meiosis. Of note, patients with r(20) fall into both groups, indicating the seizures do not result from loss of neurologically relevant genes on chromosome 20. For all autosomal rings, SNP array analysis on peripheral blood DNA showed no evidence for low-level mosaicism for monosomy, or for uniparental isodisomy in the normal cell lines, suggesting these mechanisms do not contribute to the clinical phenotype. Further studies are directed at extending these observations to additional patients, identifying the sequences involved in creation of the ring to understand the mechanism behind the associated clinical features, and elucidating the impact of ring formation on gene expression.

Missense mutations in PDE11A segregate with the disease in kindreds with testicular cancer. *A. Horvath[1], L. Korde[2], R. Libe[3], P. Osorio[1], K. Tsang[1], Y. Patronas[1], L. Tschoukani[1], L. Drori-Herishanu[1], E. Remmers[4], J. Bertherat[3], M. Greene[2], C. Stratakis[1]* 1) NICHD, NIH, Bethesda, MD; 2) NCI, NIH, Bethesda, MD; 3) Institute Cochen, Paris, France; 4) NHGRI, NIH, Bethesda, MD.

Inactivating mutations in the phosphodiesterase (PDE) 11A (PDE11A) have been implicated in predisposition to adrenocortical hyperplasia. PDE11A expression in testicular tissue were shown to be the second highest among the studied human tissues - slightly lower than in the prostate and significantly higher than in the adrenal cortex. Male sterility has been seen in the pde11a-/- mouse, and sterility in humans has been linked to TC. In this study, we sequenced the PDE11A gene coding region in 95 patients with testicular cancer (TC) from 40 unrelated families. We identified 8 different non-synonymous substitutions in a total of 23 patients from 13 families. Five of the variations were novel two we have reported before in patients with adrenocortical tumors and one was a common polymorphic variant. With the exception of one patient, all PDE11A missense variants coexisted with the disease status in the affected family members. We compared the frequency of these variants with that in a cohort of 200 unrelated controls who had been screened and found to be negative for adrenocortical diseases: only the two previously reported (R804H and R867G) that are known to be present in the general population were found in this control group. However, R804H and R867G were significantly more frequent among patients with TC than in controls (P=0.012); the combined frequency of nonsynonimous substitutions was significantly higher among TC patients (P=0.0004). Immunohistochemistry studies showed lack of expression of PDE11A protein in the tumor tissues of patients carriers of mutations in PDE11A. Ongoing studies investigate the expression of PDE11A in TC samples from these patients and the gonads of the pde11a-/- mouse. Our data suggest that PDE11A missense changes are perhaps among the many other genetic factors that may confer a predisposition to testicular tumors either within the context of the cAMP-signalling pathway or as players in a multisignaling system that controls germ cell develoment and/or tumorigenesis.

Comparison of Cardiac Pathology in Three Strains of Murine Mucopolysaccharidosis Type I: Considering the Role of Auto-immune Pathogenesis in Hurler Syndrome. *E. A. Braunlin, R. Gunther, S. L. Sandberg, R. D. Cooksley, B. Konair, C. B. Whitley* Pediatrics and Institute of Human Genetics, University of Minnesota, Minneapolis, MN.

BACKGROUND In studies of gene therapy in murine models of Hurler syndrome, we observed much longer, stable expression of -L-iduronidase (IDUA) enzyme activity in the immune-deficient strain of the IDUA knock-out mouse derived by Clarke and colleagues (NOD/SCID) in comparison to the immune-competent mouse derived by Neufeld and colleagues. HYPOTHESIS For ongoing studies that define the therapeutic response to various forms of gene therapy, we sought to characterize the untreated phenotype in both strains, and compare these to the third strain (Clarke mutation in immune-competent B6 mice) for which the cardiac anomalies have already been described (Braunlin et al, Pediatric Research 59:27-32, 2006). This may be particularly important because of the recent hypothesis that some aspects of Hurler syndrome may be auto-immune in nature. MATERIALS AND METHODS Echocardiography and histopathologic analyses of the heart were evaluated in two groups of normal and affected, 7- to 11-month-old male mice from both strains (Neufeld mutation in B6, and Clarke mutation in NOD/SCID), and the findings were compared to the previously-published studies of the Clarke mutation in the B6 background. RESULTS The comparison showed that the Clarke and Neufeld mutations on the B6 background have essentially the same echocardiographic and histopathologic abnormalities with aortic insufficiency being common in both, 10/10 and 9/13, respectively. However, mice with the Clarke mutation on the NOD/SCID background were found to have a much lower 2/12 incidence of aortic insufficiency, despite equal or greater amounts of lysosomal inclusions. CONCLUSIONS These observations in murine Hurler syndrome support the hypothesis that some aspects of the clinical phenotype are auto-immune mediated, an important factor in considering the modalities of treatment, and response to various forms of gene therapy. Comparison of the neuropathology in affected mice is currently in progress. (Supported by P01-HD032652).

Potential utility of germline genetic profiling in colorectal cancer screening: A simulation study. *S. Hawken, J. Little* Epidemiology and Community Medicine, University of Ottawa, Ottawa, Canada.

Genetic variants associated with colorectal cancer(CRC) in candidate gene and GWA studies mostly have low-penetrance. Theoretically, multiple common, low-penetrance variants collectively (not singly) could have utility in triage. Thus, while it is unlikely that a purely genetic test would be used alone, it could identify individuals at elevated genetic risk for enhanced screening surveillance. Canadian and US recommendations are for fecal occult blood (FOB) screening for all individuals 50 years and older. However compliance tends to be very low. Higher uptake rates of CRC screening have been reported in higher risk individuals (e.g.family history of disease). Hence if screening compliance in response to a positive genetic test improved to levels similar to those seen in subjects with elevated familial risk, then a substantial number of additional CRC cases might be detected early, possibly reducing CRC incidence and mortality. Using a representative population age and sex distribution from census data, 10-year age and sex specific CRC incidence rates, and varying test predictive power and compliance rates, we demonstrate that a predictive genomic test could concentrate the subjects at the highest genetic risk into a subset of the population, thus creating an enriched pool of screenees, while also simultaneously enhancing compliance to screening. For example, in the absence of a predictive genetic test, and 25% compliance with conventional screening (the current norm), 2746 cancers would get screened by the FOB test for every 1,000,000 (1M) people referred. If a strongly predictive test that concentrated 80% of cases into the top 50% of the sample were applied (i.e. 2M tests, top 1M referred to screening), and screening compliance remained at 25%, then 4398 cancers would get screened. If in addition to the strongly predictive test, the screening compliance were to improve from 25% to 40% ,7039 CRC cases would get screened, an increase of 4293 CRC cases over the baseline, for every 1M in whom screening is recommended. The majority of these cancers would be detected by the latest screening technologies.

Genome-wide genetic association study of Chronic Obstructive Pulmonary Disorder. *B. T. Webb[1,2], E. J. van den Oord[1,2], T. P. York[1,3], Y. Jia[4], E. L. Murrelle[4]* 1) Center for Biomarker Research and Personalized Medicine; 2) Department of Pharmacy; 3) Department of Human Genetics, Virginia Commonwealth University, Richmond, VA; 4) Philip Morris USA Inc. Research Center, Richmond, VA.

Chronic obstructive pulmonary disease (COPD) is a complex disease characterized clinically by airflow obstruction with cigarette smoking as primary environmental risk factor. To identify genetic risk factors for COPD, a genome-wide association study (GWAS) was performed in a sample of 200 adult smokers with COPD, 100 adult smoker controls without COPD, and 100 non-smoking controls. Using longitudinal spirometric data, basic linear unbiased predictors (BLUPs) that disentangle the pathology of COPD were calculated and analyzed as outcomes in order to increase power. The BLUPs analyzed in the GWAS included baseline lung function (Int), age related decline (AgeDec), cigarette smoke induced decline (CSDec), and an interaction between age and smoke induced decline (Age×CSDec). The minimum SNP association P values observed were 2.33×10^{-7}, 1.90×10^{-6}, 1.90×10^{-6}, and 8.5×10^{-6} for AgeDec, CSDec, Age×CSDec, and Int, respectively. False discovery rate (FDR) analysis showed the AgeDec and CSDec were enriched for significant associations. A minimum SNP specific FDR or q-value of 0.14 was found with AgeDec. A total of 33 SNPs had q-values less than 0.5 with most being associated with CSDec. Clusters of associated SNPs were found in several genes including ones which are strong candidates for COPD. The findings are generally consistent with previous evidence that immune system dysregulation is involved in the pathophysiology of COPD and that genetic differences in regulation of CS-induced inflammatory changes may influence individual disease risk. In summary, we identified several novel COPD associations in genes with functions in known COPD biological processes including cilia function / lung clearance and neutrophil activation and regulation.

Detection of sex chromosomes abnormalities; cytogenetics versus array-CGH. *M. J. Simovich, SH. L. Kang, XY. Lu, T. Sahoo, S. Lalani, C. Bacino, P. Stankewicz, A. Patel, SW. Cheung* Dept of Molecular & Human Genetics, Baylor College of Medicine.

Sex chromosome aneuploidy, microdeletions and microduplications are of specific interest due to their association with a variety of clinical syndromes. We compared the sensitivity of conventional cytogenetics versus array-CGH for detecting abnormalities of the X and Y chromosomes in samples received from January 2005 to December 2007. We identified abnormalities in 312 patients by array-CGH and in 87 by routine karyotype analysis.

	Array-CGH analysis	Karyotype analysis
X/Y aneuploidy	42	40
X/Y duplication	47	3
X/Y deletion	39	1
X/Y derivative	13	12
Multiple cell line	14	31
CNV	123	0
UCS	34	0

Array CGH identified significantly more small deletions and duplications on the X chromosome than standard G- band analysis. Our study shows that array-CGH is more sensitive than conventional chromosome analysis in detecting pathogenic copy number changes on the X chromosome.

Genome-wide map of allelic expression associated SNPs. *T. Pastinen[1,2], B. Ge[2], D. K. Pokholok[3], E. Grundberg[1,2], D. Verlaan[1,2], T. Kwan[2], V. Koka[2], K. Lam[2], L. Morcos[1,2], A. Montpetit[2], M. M. Joly[2], H. H. H. Göring[4], M. Blanchette[5], K. L. Gunderson[3]* 1) Depts of Human and Medical Genetics, McGill Univ, Montreal, Canada; 2) McGill Univ and Genome QC Innov Ctr, Montreal, Canada; 3) Illumina Inc, San Diego, CA; 4) SFBR, San Antonio, TX; 5) Dept of Computer Sci, McGill Univ.

Genetic variants altering gene expression in cis (cis-eSNPs) are an important source of phenotypic differences. We have shown earlier that such variants can be mapped using allelic expression (AE). Differential AE can be thought of as a read-out of cis-acting components of expression variance. AE mapping requires heterozygous sites in transcripts and quantitative detection of differences between heterozygote ratios in paired DNA and RNA samples. Here we have developed a first generation genome-wide map of AE associated SNPs (aeSNPs) in 50 lymphoblastoid cell lines from HapMap, using SNP genotype data from Human 1M BeadChips (Illumina). The AE was measured in unspliced primary trancripts allowing intronic SNPs to be used. Using 2SNPs for each transcript and a normalization algorithm for calculating differences in gDNA/cDNA allele ratios resulted in ~90% concordance in AE calls with Sanger sequencing. To map aeSNPs ~0.9Gb of genome, including 10K RefSeq genes, was partitioned into 80K informative windows - regions with 2 expressed SNPs in at least eight heterozygous individuals. AE was observed in 3 samples in 40% of windows, and we were able to merge 17K windows into longer expressed regions with continuous differential AE. At p<10E-6 we detected aeSNPs for 2052 RefSeqs and 1463 regions outside RefSeq annotations. A 58% overlap was observed between earlier described transcript level cis-eSNPs and aeSNPs. Conversely, aeSNPs showed converging eSNPs in 38% of cases. Complex patterns of heritable AE observed in disease and other loci underscores diversity of functional variation in human genes. Over 20 candidate regions with strong AE but no aeSNPs are candidates for novel imprinted loci. We are now pursuing AE mapping in additional samples, populations and cell types as well as developing new statistical approaches for aeSNP detection.

THE 1000 GENOMES PROJECT. *D. Altshuler[1,2,3] for the 1000 Genomes Project Consortium (www.1000genomes.org)* 1) Massachusetts General Hospital; 2) Harvard University; 3) Broad Institute of Harvard and MIT.

The first generation of genome wide association studies have demonstrated that systematic studies of genetic variation offer a general approach to map novel loci contributing to common human diseases and medical phenotypes. At only a few such loci are causal mutation(s) yet identified, however, and most heritability of common diseases remains unexplained. Thus, the next steps in genetic mapping require a more complete picture of single nucleotide and structural variants across human populations, and methods to discover rare variants with high specificity and accuracy. We have formed an international public-private consortium, The 1000 Genomes Project, to identify and make publicly available all DNA variation in the accessible genome at minor allele frequency (MAF) 1% and above. A variety of next-generation sequencing technologies are being employed and compared. The consortium includes sequencing centres, technology companies, statistical and population geneticists, ethicists, and funders. Data will be released without restriction before publication via sites at the EBI and NCBI. Cell lines are widely available for further study. Our focus in 2008 is three pilot projects: 1) To evaluate strategies to identify variants by combining data across multiple samples: low coverage whole genome sequencing of 180 individuals from the HapMap CEU, YRI and CHB/JPT populations. 2) To obtain high quality reference data for a small number of individuals, and to evaluate the tradeoffs between depth, coverage, sensitivity and accuracy: high coverage whole genome sequencing of two parent-offspring trios. 3) To explore strategies and technologies for capature and deep sequencing of functional gene region: deep sequencing of ~1000 genes in ~1000 samples,. At the time of submission 600 Gb of data (over 200-fold coverage of the human genome) have been deposited and are being processed for public release. We anticipate that data collection for the three pilots will be complete by ASHG, and propose to to present the design, rationale, initial results and analysis of the 1000 Genome Project data.

Development of Methods to Improve Base Calling for DNA Resequencing Arrays. *W. Wang[1], P. Shen[1], M. Yu[1], T. Klopstock[2], R. Horvath[3], C. Palm[1], L. Pique[4], I. Schrijver[4], D. Cutler[5], M. Mindrinos[1], R. Davis[1], T. Speed[6], C. Scharfe[1]* 1) Stanford Genome Technology Center, Stanford U., Palo Alto, CA, USA; 2) Dept of Neurology, Ludwig Maximilians U., Munich, Germany; 3) Friedrich-Baur-Inst, Ludwig Maximilians U., Munich, Germany; 4) Dept of Pathology and Pediatrics, Stanford U., Stanford, CA, USA; 5) Dept of Human Genetics, Emory U., Atlanta, GA, USA; 6) Dept of Statistics, UC Berkeley, Berkeley, CA, USA.

The identification of rare DNA variants (1-3%) in disease candidate genes across many medical cases remains challenging. Medical resequencing (MRS) arrays custom-designed using Affymetrix GeneChip technology provides a strategy for DNA sequencing of up to 300kb per array. For diploid genomes, available base calling tools are limited and based on pixel-level data and likelihood ratio tests. Previous success with RMA (robust multi-array analysis) for gene expression and SNP arrays suggest that the analysis of probe-levels across multiple arrays improves the signal detection. Here we take advantage of these ideas and develop novel methods for MRS array analysis to account for the following: 1) The amplification of individual DNA fragments leads to heterogeneity in data quality; 2) The classification of genotypes is based on query of all four possible nucleotides on both strands; and 3) The probes move along the sequence one base at a time, creating a continuous decrease of the neighboring probe intensities around a SNP location. Our custom-designed MRS arrays contain all exons and intronic splice-sites of 39 nuclear-encoded mitochondrial genes that are candidates for hereditary optic neuropathies. We Sanger-sequenced four genes for reference. Our analysis of 40 MRS samples using established algorithms (i.e. GSEQ, RATools) in comparison to our methods improves the detection of non-variants, homozygote and heterozygote variants with reduction in false positive and false negative base calls. Our efforts lead to improvements of laboratory protocols for array processing and represent steps towards our goal to perform cost-efficient candidate gene resequencing in human disorders.

A genome-wide association study of 1,000 Han-Chinese with Type 2 diabetes mellitus cases vs. 1,000 controls residing in Taiwa. *J. Y. WU[1,2], F. J. TSAI[2], C. C. CHEN[2], P. CHEN[1], C. F. YANG[1], C. H. CHEN[1], Y. M. LIU[1], C. F. SHIU[1], C. S. J. FANN[1], Y. T. CHEN[1]* 1) Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan; 2) China Medical University Hospital, Taichung, Taiwan.

Background Type 2 diabetes mellitus (T2DM) is the forth leading cause of death in Taiwan. The prevalence of DM in Taiwan increases from 4.63% in 2001 to 4.69% in 2003 and the mortality from diabetes mellitus has almost doubled over the past ten years. This study aims to identify genetic components associated with T2DM, which is significant for the Han-Chinese, account for 98% of the Taiwan population.

Methods A total of 1,000 Han-Chinese T2DM patients and 1,000 random controls were genotyped with the Illumina Hap550duov3 chip as well as the deCode CNV chip. Type II diabetes mellitus was diagnosed using the American Diabetic Association Criteria. All 1000 patients were recruited from the China Medical University Hospital, Taichung, Taiwan. DNA of the 1,000 random controls was selected from the Taiwan Han-Chinese Blood and Cell Bank, Academia Sinica, Taiwan. We excluded SNPs from further analyses by three major criterions: (1) missing data rate>5%, (2) missing data rate>1% for SNPs with a minor allele frequency < 5%, and (3) p-value of Hardy-Weinberg Disequilibrium test <10-7. Haplotpye (multi-point) analyses were applied to summarize regions with multiple significant points.

Results A total of 517,401 SNPs (92.36%) passed the quality control filter with an average call rate of 99.91% and subjected to genome-wide association analysis. None of the SNPs were significant at = 10-7. However, 10 SNPs and 4 regions showed positive association (10-7 < p < 10-5); none of these were located at or near the points previously reported by others.

Summary We have identified novel SNPs/regions associated with Chinese patients with T2DM. Our study indicated the heterogeneity of type 2 diabetes mellitus between the Asian and Caucasian populations.

Association of 9p21 SNPs with premature atherosclerosis in the Pathobiological Determinants of Atherosclerosis in Youth (PDAY) study. *T. D. Howard[1], Y. Liu[1], C. D. Langefeld[1], K. Konvicka[2], E. Beilharz[2], C. Johnson[3], J. E. Hixson[4], J. I. Rotter[5], Y.-D. I. Chen[5], D. A. Troxclair[6], G. T. Malcom[6], J. P. Strong[6], D. M. Herrington[1]* 1) Wake Forest Univ Health Sciences, Winston-Salem, NC; 2) Perlegen Sciences, Inc, Mountain View, CA; 3) Univ of Washington, Seattle, WA; 4) Univ of Texas Health Science Center, Houston, TX; 5) Cedars-Sinai Medical Center, Los Angeles, CA; 6) LSU Health Sciences Center, New Orleans, LA.

Several groups have reported an association of SNPs on 9p21 (rs10757274 & rs2383206) with coronary artery disease and MI. This association was replicated in several adult Caucasian populations with coronary disease based on clinical events, angiography or coronary CT. To increase the pathophysiologic specificity of this association and to explore it in other ethnic groups, we evaluated 1065 individuals from PDAY. PDAY consists of individuals ages 15-34 that died of non-cardiovascular causes. A standardized autopsy protocol quantified the percent surface area involvement of raised atherosclerotic lesions in the abdominal aorta, thoracic aorta, and right coronary artery (raised lesion score). 357 cases (166 African-Americans, 191 Caucasians) with the highest total age-adjusted raised lesion scores were frequency matched on age, race, and gender with 708 controls (331 African-Americans, 377 Caucasians) with the lowest total raised lesion scores. Genotypic distributions were consistent with HWE in controls (p=0.21, 0.17), but not cases (p=0.054, 0.0072). In Caucasians the ORs under an additive model for rs10757274 and rs2383206 were 1.27 and 1.29 (nominal p=0.037 & 0.049, respectively), with the G allele for both SNPs associated with increased risk for extensive premature disease. In African-Americans, borderline evidence for association was observed (nominal p=0.052 & 0.067), but the direction of the association was reversed (OR 0.72 & 0.78). **Conclusion:** These data suggest that rs10757274 and rs2383206 are associated with premature and pathologically-defined atherosclerosis in Caucasians, but not African-Americans, and that a genetic predisposition for atherosclerosis may be the underlying pathological determinant of the previously reported associations with CVD.

Genome wide association identifies an asthma susceptibility locus on chromosome 1q31 in both Caucasian and African American children. *P. Sleiman[1], M. Imielinski[1], J. P. Bradfield[1], K. Annaiah[1], S. Willis-Owen[2], N. M. Rafael[3], S. Michel[4], C. E. Kim[1], R. Grundmeier[5], J. Allen[6], J. Spergel[7], J. Christie[8], M. Kabesch[4], M. F. Moffatt[2], M. M. Grunstein[5], K. C. Barnes[3], M. Magnusson[9], S. F. A. Grant[1,9], H. Bisgaard[10], H. Hakonarson[1,6,9]* 1) Center for Applied Genomics, Children's Hospital of Philadelphia; 2) Imperial College London, UK; 3) Johns Hopkins University, Baltimore; 4) Ludwig Maximilian University, Munich, Germany; 5) Dept of Bioinformatics, CHOP; 6) Division of Pulmonary Medicine,CHOP; 7) Division of Allergy and Immunology, CHOP; 8) Division of Pulmonary Medicine, University of PA School of Medicine; 9) Dept of Pediatrics, CHOP; 10) Department of Health Sciences, University of Copenhagen.

Asthma is a heterogeneous disease of complex etiology. We carried out a GWA study utilizing over 545,000 SNPs in 2236 pediatric asthma patients, including 569 North Americans of European ancestry and 1667 African Americans. We describe a locus on chromosome 1q31 containing multiple common variants that are highly and reproducibly associated with pediatric onset asthma in both Caucasians of European ancestry and African Americans. The most highly associated SNP in the Caucasians was rs2134409 (MAF 0.146 cases and 0.22 controls, OR = 0.604 [95% CI 0.47 - 0.78], P-value 7.8x10-8). In African Americans rs2134409 was highly significant (P=3.6x10-05) however, the most significant was rs1747815 (MAF 0.51 cases and 0.28 controls, OR = 1.86 [95% CI 1.46 - 2.37] P-value 2.9 x10-7). In independent replication studies, the 1q31 locus yielded significant association with asthma in 379 North Americans of European ancestry (rs2134409 P=0.032); and an additional 918 pediatric cases of Northern European ancestry (r2 rs2134409-rs2111931 0.83, rs2111931 P=1.94 x10-04). Analysis of the associated interval indicated that all the associated SNPs mapped to a block of high LD encompassing DENND1B and the 3 end of CRB1 in four independent studies of Caucasians of European decent and African Americans. The associated interval contains DENND1B, a natural asthma candidate gene known to interact with the TNF receptor, a well established asthma pathway candidate.

A computational method for predicting the functional effect of missense mutations. *S. Sunyaev[1], S. Schmidt[2], L. Peshkin[3], A. Adzhubei[1], A. Gerasimova[4], A. Kondrashov[4], V. Ramensky[5]* 1) Div Genetics, Dept Medicine, Brigham & Women's Hospital and Harvard Medical School, Boston MA, USA; 2) Steffen Schmidt, Max Planck Institute for Developmental Biology, Tuebingen, Germany; 3) Dept Systems Biology, Harvard Medical School, Boston MA, USA; 4) Life Science Institute, University of Michigan, Ann Arbor MI, USA; 5) Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russia.

Rapid development of sequencing technology puts to the forefront the need to interpret DNA sequence information. Sequencing of phenotyped clinical populations is widely anticipated to replace genotyping in studies aiming at finding genes underlying human complex diseases. Clinical genetic diagnostics by sequencing is increasingly important in guiding therapeutic intervention and providing counseling to family members of patients with monogenic and oligogenic diseases, although interpretation of results of diagnostic sequencing is sometimes problematic because sequencing of patient cohorts discovers many variants of unknown significance (VUS). These developments create an unprecedented demand for development of computational methods for interpretation of nucleotide and amino acid changes, and for predicting the effect of mutations and polymorphism on molecular function, fitness and phenotype. We developed and extensively tested a new method PolyPhen2 for predicting the functional effect of human missense mutations. The method combines comparative sequence analysis, analysis of protein 3D structure and database annotations. We created a new highly accurate automated multiple sequence alignment pipeline and designed several novel features predictive of the effect of mutations. We employed a number of machine learning techniques to select the best set of features and to generate prediction rules. These developments resulted in a greatly increase sensitivity and specificity of predictions as evident from tests on several datasets. We also analyzed applicability of the method in the setting of a clinical genetic diagnostics lab. The method has been implemented in publicly available software.

ITMAT/Broad/CARE (IBC) candidate gene genotyping platform identifies genes newly associated with HDL cholesterol. *D. J. Rader[1], A. Edmondson[1], I. Stylianou[1], S. DerOhannessian[1], A. Khera[1], M. Wolfe[1], B. Keating[1], M. Reilly[1], M. Li[2]* 1) University of Pennsylvania, School of Medicine, Institute for Translational Medicine and Therapeutics, PA, USA; 2) Department of Biostatistics & Epidemiology, University of Pennsylvania School of Medicine, PA, USA.

High density lipoprotein cholesterol (HDL-C) concentration is inversely associated with coronary artery disease (CAD) and is a highly heritable trait. There are a large number of candidate genes based on biology that have never been tested for genetic association with HDL-C. The ITMAT/Broad/CARE (IBC) candidate gene array is a custom 50K cardiovascular disease SNP array developed in a multi-institutional collaboration. It aims to comprehensively assess the genetic diversity within genes (> 2100) and pathways underpinning primary and secondary vascular disease processes. More than 100 HDL-C candidate genes were chosen for inclusion on this array based on known biology, mouse genetics, and HDL proteomics studies. We used the IBC array to genotype an HDL case-control cohort (600 cases, HDL > 90th percentile; 600 controls, HDL < 30th percentile) to interrogate the association of candidate genes with HDL-C. Many previously validated HDL-C associations appeared very strongly in this study, including CETP (p = 9.4 x 10-14), LPL (p = 5.9 x 10-6), and ABCA1 (p = 1.3 x 10-4). Multiple other HDL candidate genes without prior human association data were found to be significantly associated with HDL-C levels. Replication of HDL-C associations was performed in a separate cohort (PennCATH) also genotyped using the IBC array and significant SNPs were further tested for their association with angiographic CAD. The IBC array will make possible the testing of the association of a large number of candidate genes with relevant cardiovascular phenotypes and has already established the novel association of several genes with variation in HDL-C and the relationship of the same genes to CAD.

Candidate gene association study of *DLGAP3* in Tourette Syndrome patients. *J. M. Scharf[1,2,3,4], J. Fagerness[1,2], B. Gunnell[1,2], S. E. Stewart[1,2], D. L. Pauls[1,2], Tourette Syndrome International Consortium for Genetics* 1) Psychiatric Neurodevelopmental Genetics Unit, Center for Human Genetics Research, Massachusetts General Hospital, Boston, MA; 2) Dept. of Psychiatry, Massachusetts General Hospital, Boston, MA; 3) Dept. of Neurology, Massachusetts General Hospital, Boston, MA; 4) Dept. of Neurology, Brigham and Women's Hospital, Boston, MA.

OBJECTIVE: To test the hypothesis that *DLGAP3* (human ortholog of mouse *Sapap3*) is associated with Tourette Syndrome (TS) using a family-based association approach. BACKGROUND: TS is one of the most heritable non-Mendelian neuropsychiatric disorders, yet no definitive TS susceptibility gene has been identified to date. Recently, a targeted deletion of mouse *Sapap3* (*Sapap3-/-*) was demonstrated to have a compulsive grooming disorder similar to human obsessive-compulsive disorder and trichotillomania, two conditions believed to be genetically related to TS. DESIGN/METHODS: Subjects included 375 complete trios with TS as well as 20 additional affected siblings (395 total affected). Diagnoses were made by DSM-IV-TR criteria and confirmed by a consensus of TS clinical investigators. Common tagSNPs (5% minor allele frequency) were selected from the HapMap Phase II, SNPper and dbSNP databases spanning *DLGAP3* including 40kb of flanking sequence that contained additional highly conserved mammalian sequences. Genotyping of tagSNPs was performed by primer extension and mass spectroscopy detection methods. Family-based association analyses were performed using the program FBAT. RESULTS: Preliminary analysis of a subset of tagSNPs demonstrated no association between *DLGAP3* and TS. Analysis of the complete tagSNP set is currently in progress. CONCLUSIONS: *DLGAP3* remains an intriguing candidate gene for TS. Complete analysis of this sample as well as future genomewide association studies will hopefully provide more definitive evidence for association between *DLGAP3* and TS.

Detection of rare male cells surrounded by female cells using automated slide scanning. *S. Ayub, O. Samassekou, MC. Gregoire, M. Gadji, A. Emad, E. Bouchard, R. Drouin* Genetics, Dep of Pediatrics, Fac Med Health Sci, Univ Sherbrooke, Sherbrooke, Quebec, Canada.

Introduction- Fetal male cells are found in maternal circulation at a rare frequency of 2-6 cells/ml of maternal blood. These male cells that are a source of developing a non-invasive prenatal diagnosis, are identified using a cell-type independent marker (XY). Objectives- To alleviate the burden of manual scanning we are in the process of validating Ikoniscope - an automatic slide scanning apparatus, by diluting XY cells in XX cells in a proportion similar to the number found in the peripheral blood of a female during 18th to 22nd weeks of pregnancy. Methodology- The method involves spreading 1-10 XY cells on clean slides, staining with Giemsa, counting the cells, imaging, then spreading XX cells followed by Fluorescence In Situ Hybridization (FISH) using X and Y probes. The slides are loaded on Ikoniscope and the pictures taken by the machine are compared with the ones taken before FISH. So far, forty slides have been prepared and automatically scanned. Results- For each slide, there were approximately 3000 optical fields at 20X and on an average 5 pictures at high magnification (100X). An analysis of the pictures taken by the Ikoniscope reveals that it is missing around 50% of the cells. Around 90% of the times, cells were missed due to out of focus fields and the rest were missed due to FISH inefficiency. Cells at the edge of two adjacent fields were also missed. Conclusion- Since automatic slide scanning is less cumbersome and less time consuming than manual scanning, we are currently working on optimization of the scanning. Once the validation of automatic scanning is completed it would revolutionize the slide scanning for detection of fetal cells in peripheral blood of pregnant women.

Association of sequence variant rs10757278 on 9p21 with intracranial aneurysm. *R. Deka[1], D. Koller[2], D. Lai[2], S. R. Indugula[1], G. Sun[1], D. Woo[1], L. Sauerbeck[1], R. Hornung[3], E. Sander Connolly[4], C. Anderson[5], G. Rouleau[6], I. Meissner[7], J. Bailey-Wilson[8], J. Huston[7], R. Brown[7], C. Langefeld[9], T. Foroud[2], J. Broderick[1], FIA Investigators* 1) University of Cincinnati School of Medicine, Cincinnati, OH; 2) Indiana University School of Medicine, Indianapolis, IN; 3) Cincinnati Children's Hospital Medical Center, Cincinnati, OH; 4) Columbia University, New York, NY; 5) University of Sydney, Sydney, Australia; 6) Notre Dame Hospital, Montreal, Canada; 7) Mayo Clinic, Rochester, MN; 8) Natioanl Human Research Institute, Baltimore, MD; 9) Wake Forest University, Winston-Salem, NC.

Several studies have recently reported association of two common variants on 9p21, rs10757278 and rs10811661, with coronary artery disease (CAD) and type 2 diabetes (T2D). A subsequent study in multiple populations reported that the G allele in rs10757278 was associated with abdominal aortic aneurysm (AAA) and intracranial aneurysm (IA) in addition to CAD (Helgadottir et al. Nat Genet 2008). We typed this variant to test for association with IA in a sample of 270 cases and 281 controls from the Familial Intracranial Aneurysm (FIA; www.FIAStudy.com) study. We found significant association of the G-allele with FIA cases (p=0.0123). We also found significant genotypic association (p=0.0367), with an excess of GG homozygotes and AG heterozygotes as observed previously (Helgadottir et al.). Our results provide nominal replication that a risk factor in the 9p21 region influences the risk of IA. Funded by R01NS039512.

A genome-wide association study of 1,000 Han-Chinese bipolar I cases vs. 1,000 controls residing in Taiwan. *M. T. M. Lee, C. H. Chen, C. H. Lin, C. C. Chang, L. C. Chang, C. F. Li, H. T. Wang, J. Y. Wu, C. S. J. Fann, Y. T. Chen, A. T. A. Cheng, Taiwan Bipolar I Disorder Consortium* Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan.

Background Bipolar I disorder is a complex disease with high heritability. Most of the previous gene-mappings were based on small number of families in western populations and their results were not replicated by a large-scale population-based study conducted in UK. Hence, more population-based studies in other populations are in demand. In this study, we conducted a genome-wide association study to detect genes susceptible to bipolar I disorder in the Taiwan Han-Chinese. **Methods** A total of 1,000 Han-Chinese bipolar I patients and 1,000 random controls were genotyped with the Illumina Hap550duov3 chip as well as the deCode CNV chip. The patients were recruited through the Taiwan Bipolar I Disorder Consortium and the DNA of random controls was extracted from the Taiwan Han-Chinese Blood and Cell Bank. Bipolar I phenotype assessment was conducted using a Chinese version of the SCAN (WHO Schedules for Clinical Assessment in Neuropsychiatry). We excluded SNPs from further analyses by three major criterions: (1) missing data rate >5%, (2) missing data rate >1% for SNPs with a minor allele frequency <5%, and (3) p-value of Hardy-Weinberg Disequilibrium test <10-7. Multi-point analyses were applied to summarize regions with multiple significant points. **Results** A total of 517,015 SNPs (92.29%) passed the quality control filter with an average call rate of 99.91% and subjected to genome-wide association analysis. Two SNPs were identified with significant signals ($p < 10\text{-}7$) while 11 SNPs and 3 regions showed positive evidence ($10\text{-}7 < p < 10\text{-}5$); none of these have been reported previously. Furthermore, a few sites have higher frequencies of copy number variations in cases than in controls; additional genotyping using Affymetrix SNP 6.0 chip on 200 cases and 200 controls confirmed the copy number variations. **Summary** We have identified novel SNPs/regions associated with Chinese patients with bipolar I disorder. Our study indicated the heterogeneity of bipolar I between the Asian and Caucasian populations.

A genome-wide association screen for loci influencing homocysteine levels. *L. Almasy[1], M. Carless[1], J. Curran[1], T. D. Dyer[1], H. H. H. Göring[1], M. P. Johnson[1], J. M. Soria[2], J. W. MacCluer[1], E. K. Moses[1], J. Blangero[1]* 1) Department of Genetics, Southwest Foundation for Biomedical Research, San Antonio, TX; 2) Institut de Recerca de l'Hospital de la Santa Creu i Sant Pau, Barcelona, Spain.

Homocysteine (Hcy) levels are a well-known risk factor for venous thrombosis, myocardial infarction, stroke, congestive heart failure, osteoporotic fractures and Alzheimers disease. We performed a genome-wide association (GWA) screen for loci influencing Hcy levels in 858 individuals in extended pedigrees from the San Antonio Family Heart Study. Hcy levels were measured using kits from Genzyme Diagnostics and values were log transformed prior to analyses. SNP genotypes were obtained using the Illumina HumanHap550 BeadChip. Measured genotype association analyses were conducted using SOLAR.

The heritability of Hcy levels in this sample was 0.49 ($p = 1.5 \times 10^{-32}$) and Hcy levels increased with age and were higher in males and in cigarette smokers. One SNP in the methylenetetrahydrofolate reductase (MTHFR) gene, rs1801133, gave genome-wide significant association results ($p = 2.5 \times 10^{-9}$), confirming previous linkage and association studies tying this gene to Hcy levels. Two SNPs in the region of the leucine rich repeat transmembrane neuronal 4 gene (LRRTM4) and one SNP in the region of the mitogen-activated protein kinase kinase kinase 9 gene (MAP3K9) provided suggestive evidence of association ($p < 1.9 \times 10^{-6}$) at levels expected to occur by chance less than once per GWA scan. Nine SNPs in the region of the protocadherin 7 gene (PCDH7) showed association results that just missed this cut-off for suggestive signals ($p = 7.2 \times 10^{-6}$ to 3.5×10^{-6}). We were also able to confirm associations of Hcy levels with the nicotinamide N-methyltransferase gene (NNMT) previously identified through linkage analyses in a Spanish population by the Genetic Analysis of Idiopathic Thrombosis (GAIT) study ($p = 0.005$).

We are currently in the process of expanding the GWA sample size and examining correlations between Hcy levels and RNA expression levels of the genes implicated in the GWA scan.

Seizures in urea cycle disorders (UCDs): an under-recognized symptom in patients outside of the acute metabolic phase. *N. Zecavati, U. Lichter-Konecki, R. Singh, J. Crawford, R. Seltzer, A. Gropman, Children's National Medical Center, George Washington University* Neurology, Children's National Medical Center, Washington, DC.

Objective: To investigate the frequency of seizures in UCDs outside of coma. Methods: We performed a retrospective chart review of patients presenting to our institution over the last 30 years who were ultimately diagnosed as having a UCD and either a single seizure or epilepsy. We identified five patients and queried charts for UCD type, age of diagnosis, timing of first seizure relative to diagnosis, seizure type, EEG pattern, and metabolic studies. Results: There were 2 females and 3 males. Two had ornithine transcarbamylase deficiency (OTC), 3 had arginosuccinic aciduria (ASA), and 4/5 had generalized seizures. In 3/5 cases, seizures occurred after UCD diagnosis but were initially unrecognized. Barriers to diagnosis included behavioral disturbances such as ADHD, OCD, and ODD, which were present in 4/5 patients. Most patients were treated with Keppra or Lamictal with good seizure control. Conclusions: UCDs are a common group of inborn errors of metabolism. In the acute setting, the neurological effects of increasing CNS ammonia include anorexia, vomiting and mental status changes. Progressive CNS dysfunction, reflecting ammonia-induced cell swelling, leads to lethargy, ataxia, seizures, hypothermia and coma. While seizures are known to occur in the acute metabolic phase, the incidence of seizures prior to diagnosis is unknown and thought to be rare. Impediments to diagnosis include comorbid CNS dysfunction accompanying UCDs. Based on this, a low level of suspicion for seizures in children with UCDs should be entertained, especially in the face of changes in behavior or attention without metabolic changes. Use of AEDs may be challenging given underlying metabolic disorder.

In vivo evaluation of chromosomal damage by 4 Gy of ionizing radiation in the presence of curcumin and amifostine. *A. Corona-Rivera, J. E. Franco-Pérez, R. Zepeda-Mora, E. G. Orozco-Arizaga, Y. Martínez-Martínez, C. L. Guzmán-Gutierrez, C. Ortega de la Torre, L. Bobadilla-Morales* Universidad de Guadalajara, Departamento de Biología Molecular y Genómica, Instituto de Genética Humana "Dr. Enrique Corona Rivera", Laboratorio de Citogenética Genotoxicidad y Biomonitoreo.

Ionizing radiations induce chromosome aberrations in a dose dependent manner. In Chinese hamster ovary cell cultures curcumin contributed positively to the gamma-radiation-induced chromosome aberration. A previous study in mice showed radioprotective effect of curcumin evaluated by micronuclei, which was presented 2 hrs before moderate ionizing radiation exposition. Longer exposition curcumin periods may be more effective. Amifostine is a known radioprotector. The aim of this work is to test curcumin and amifostine by chromosome aberrations induced by 4 Gy ionizing radiation in a murine model. We evaluated 30 Balb-C mice, grouped as follows: I, control; II, radiation 4 Gy; III, radiation 4 Gy + curcumin 1%; IV, radiation 4 Gy + amifostine 400 mg/kg (IP, 1 hr before radiation exposure. Mice were fed with curcumin 5 days before radiation exposure. 48 hrs after radiation exposure, the mice were sacrificed to obtain chromosomal preparations. Chromosomal aberrations were scored in around 100 metaphases considering breaks, gaps, rings, dicentrics and acentric fragments. We found that total aberrations and percentage of damaged cells were significantly increased in radiated group versus control, apparently due to an increase of chromatid breaks (Dunetts T3). When curcumin or amiphostine were present in radiated groups, there was no statistical difference versus control (Dunetts T3). Apparently, curcumin and amiphostine were able to diminish the damage due to 4 Gy of radiation exposition.

Variation in the DC-SIGN gene and susceptibility to HHV-8 infection and Kaposi's Sarcoma in HIV-positive men enrolled in the Multicenter AIDS Cohort Study (MACS). *R. S. Bosko, R. E. Ferrell, C. R. Rinaldo, J. J. Martinson, Multicenter AIDS Cohort Study (MACS)* Grad. School of Public Health, University of Pittsburgh, Pittsburgh, PA.

Human Herpesvirus 8 (HHV8) is the causative agent of Kaposi's Sarcoma (KS) in HIV-1 positive individuals. HHV8 is now known to use the C-type lectin molecules DC-SIGN and DC-SIGNR as major receptors in the infection of its target cells, but little is known at present about the extent to which host genetic variation affects virus binding and invasion, and the subsequent progression to KS in HHV8 infected HIV-1 positive individuals.

We determined the extent of sequence variation in the DC-SIGN gene in a sample of 417 men enrolled in the Multicenter AIDS Cohort Study. All men were HIV-1 positive, and were divided into three groups: HHV8+/KS+, HHV8+/KS-, and HHV8-/KS-. Case and control groups were matched based on exposure risk. PCR amplicons containing SNPs were detected by denaturing HPLC, and the SNP was identified by resequencing. The entire coding, promoter, and downstream regulatory regions of DC-SIGN were screened for each individual. A total of 49 SNPs were detected, of which 20 were present with a Minor Allele Frequency (MAF) >5%. Haplotype distributions were obtained using PHASE software.

Few SNPs were found that altered the amino acid sequence of DC-SIGN, and those that were present did not differ in frequency between the case and control groups. Most of the SNPs found were located in promoter and regulatory regions, suggesting that quantitative variation in DC-SIGN levels may be common. Haplotypes based on common SNPs did not vary significantly between the three groups, suggesting that variation in DC-SIGN is not a major determinant of HHV8 infection or disease pathogenesis. Extended haplotypes including rare SNPs were significantly different between the groups (p0.05), but were low in frequency overall and are not a common determinant of KS pathogenesis in the MACS.

The Association between Sequence Variation in the Serotonin Transporter Gene and Obsessive-Compulsive Disorder Symptom Dimensions. *P. D. Arnold[1], J. Beneteau[2], T. Sicard[3], E. Burroughs[4], J. L. Kennedy[3], M. A. Richter[4]* 1) Dept Psychiatry, Hosp Sick Children, Toronto, ON, Canada; 2) Dept Psychology, MacMaster University, Hamilton, ON, Canada; 3) Neurogenetics Section, Centre for Addiction and Mental Health, Toronto, ON, Canada; 4) Anxiety Disorders Clinic, Centre for Addiction and Mental Health, Toronto, ON, Canada.

Obsessive-compulsive disorder (OCD) is a disabling psychiatric condition with strong genetic determinants. Factor analytic studies of OCD symptoms have consistently demonstrated 4 to 5 symptom dimensions which may have distinct genetic risk factors, however most candidate gene studies of OCD have failed to examine symptom dimensions. Purpose: We set out to determine whether OCD symptom dimensions are associated with variants in the serotonin transporter (5-HTT) gene. Methods: A total of 70 parent-proband trios ascertained through an adult with OCD were genotyped for 4 polymorphisms: a promoter insertion/deletion polymorphism (5HTTLPR), rs25531 (a SNP in the long allele of 5HTTLPR), a tandem repeat polymorphism in intron 2 (STin2), and rs1042173 (a SNP in the 3-untranslated region). Analyses were performed using the Family-Based Association Test (FBAT) on the following phenotypes: OCD diagnosis and 5 symptom factor scores derived through principal components analysis of lifetime symptoms collected using the Yale-Brown Obsessive-Compulsive Scale (YBOCS) checklist. Results: No significant associations were found with OCD diagnosis. After correcting for multiple comparisons, the A allele of rs1042173 was associated with higher scores on the contamination symptoms dimension ($z=3.01$, $p=0.003$). The combination of the long allele of 5HTTLPR and the A allele of rs25531, which has been shown to increase gene expression, was significantly associated with higher symmetry scores ($z=3.06$, $p=0.002$). Conclusion: These results provide further evidence that 5-HTT variation may be implicated in OCD, and suggest that quantitative symptom dimensions may be more powerful than qualitative diagnostic categories for detecting susceptibility genes. It is hoped that the identification of susceptibility genes will lead to improved OCD diagnosis and treatment.

Pairing of homologous chromosome regions correlates with the frequency of mitotic recombination. *I. Tereshchenko[1,2], L. Serrano[1,2], Y. Chang[1], N. Goldsmith[1], S. Buyske[1], C. Ayala[1], C. Shao[1], J. Tischfield[1]* 1) Rutgers Univirsity, Piscataway, NJ; 2) equal contributors.

We have developed an in vivo mouse assay for loss of heterozygosity (LOH) and have shown that the major mechanism in somatic cells is mitotic recombination (MR). In interphase cells, chromosomes occupy non-overlapping, discreet areas called territories; the arrangement of territories and specific chromosomal sub-regions has been correlated with DNA replication, transcription and repair. Since MR requires contact between homologous regions of chromosome homologs, we tested the hypothesis that the spatial arrangement of homologous regions regulates the frequency of MR. We analyzed primary fibroblasts of heterozygous Aprt K.O.($Aprt^{+}/Aprt^{neo}$) mice. Physical loss of $Aprt^{+}$ was confirmed by allele-specific PCR. STS and SNP markers mapped the location of MR crossover events. Regions with high and low recombination rates showed marked differences in DNA sequence composition. The high frequency region was enriched with simple repeats, transposable elements, and DNA binding sites for regulatory molecules essential for long-range chromosomal interaction. The spatial organization of chromosome territories and sub-regions was analyzed by FISH and 3D reconstruction techniques. The position of the high recombination region is distinct from the spatial positions of flanking segments, looping out from proximal (<4Kb) chromatin regions, suggesting that its position is locally regulated. Homologous chromosomal regions with high recombination rates were spatially paired (<0.6 m apart) in ~4% of exponentially growing cells. This pairing was significantly higher than that observed for low recombination regions (0.5%). Pairing of homologous regions is distinctly non-random throughout the cell cycle. Our results suggest that the relative physical proximity of homologous chromosomal regions may determine the probability of a mitotic recombination event and support the hypothesis that nuclear architecture regulates the frequency at which a particular chromosomal region undergoes MR leading to LOH.

Differential association of the CYP3A4 alleles and phynotypes with the onset of breast tumors. *D. McDaniel[1,2], T. Thurber[2], A. Lewis[1], X. Zhou[1], S. Bigler[3], R. Vance[2], W. Barber[1,2]* 1) Dept Surgery; 2) School of Medicine; 3) Department of Pathology, Univ Mississippi Medical Ctr, Jackson, MS.

Background: Greater than 50% of breast tumors are hormone-dependent and breast tissue is a local source for both estrogen and estrogen receptors. Estrogens are produced through the activity of Cytochrome P450 aromatase. Particularly, P450 3A enzymes are involved in the metabolism of several drugs, including Tamoxifen, used in breast cancer therapy. Effective treatment of breast tumors depends on identifying the tumor at the earliest stage. We hypothesized that CYP 3A4 allele variant and the amounts of the CYP 3A4 activity might have a potential relationship with the degree of tumor differentiation, histological grading and hormone receptors. Methods: Patients who had undergone biopsy procedures for diagnosis or for partial or radical mastectomy were recruited in this study. Clinical diagnosis was obtained from pathology database. For genotype detection (AG at position -290 of the CYP3A4 gene produce AA, AG and GG genotypes), SNP analysis was used to identify the CYP3A4 gene variants in peripheral blood nucleated cells (PBNCs). Results: Expression levels of mRNA transcripts were determined by RT-PCR. The AA genotype was identified in 47% of patients with malignant tumors as compared with 33% of patients with benign tumors. The GG genotypes were significantly increased in African American patients requiring breast biopsy as compared with healthy controls, and 71.4% patients with benign tumors carrying the GG genotypes had the pathology diagnosis of fibroadenoma. A majority of patients (58%) with stage II tumor diagnosis carried the GG genotype as compared with 20% and 29% for AA and AG genotypes respectively. Estrogen receptors were inversely associated with the GG genotypes, while the HER-2/neu receptors were increased in patients with AG and GG genotypes. The 3A4 mRNA transcript appears to be higher in PBMCs of patients with benign tumors compared to malignant tumors, suggesting that increased hormone metabolism by CYP 3A4 may mean a better prognosis for breast tumors. Conclusion: Genotype analysis may predict likelihood of tumor development and tumor stages.

Identification of two novel sequence variants in two X-linked mental retardation genes: ATRX and RSK2. *O. I. Batista[1,4,5], T. Maher[1], A. Milunsky[1,2], J. M. Milunsky[1,2,3]* 1) Center for Human Genetics; 2) Department of Pediatrics; 3) Department of Genetics and Genomics, Boston University School of Medicine, Boston, MA; 4) Obbj Gendiagnostik; 5) Universidad Autónoma de Chiriquí, Provincia de Chiriquí, Panamá.

ATRX and RSK2 are two genes whose mutations cause X-linked mental retardation (XLMR), a common cause of inherited intellectual disability with an estimated prevalence of 1/1000 males. Alpha thalassemia/mental retardation syndrome, X-linked (ATRX), is a severe form of XLMR associated with urogenital abnormalities, facial dysmorphism and mostly with alpha thalassemia. Mutations in the ATRX gene have been shown to underlie this condition. Coffin-Lowry syndrome (CLS) is caused by mutations in the RSK2 gene. CLS affected males show severe MR with characteristic dysmorphism, most notably affecting the face and hands. A 4-year-old boy with a clinical history of mental retardation and microcephaly and a 7-year-old boy with mental retardation and dysmorphic features were referred to our center for sequence evaluation of the genes ATRX and RSK2, respectively. Two sequence variants, previously not reported, were detected. A hemizygous 3 bp deletion (IVS9-3_IVS9-1delAAG) which includes the splice acceptor site, was found in the ATRX gene. This alteration is predicted to cause a frameshift and protein truncation. Protein products of altered size could be determined by further analysis of the probands cDNA. The second variant, identified in the RSK2 gene, is a missense change c.777T>G(p.F259L). This variation results in a non-synonymous substitution of phenylalanine by leucine in the N-terminal kinase domain of the RSK2 protein and, consequently, is likely to produce a deleterious effect. Complementary review of the RSK2 protein sequence using SIFT and PolyPhen tools supports the hypothesis of a deleterious effect caused by the identified F259L change. Further clinical and genetic analysis of the family's probands is recommended in order to clarify the clinical significance of both sequence variants reported here.

Molecular and cytogenetic characterization in a case of terminal deletion 7q36 and literature review. *R. Drouin, S. Ayub, M. Gadji, S. Cote, K. Krabchi* Dept Ped, Fac Med Hlth Sci, Univ Sherbrooke, Sherbrooke, PQ, Canada.

Introduction- Partial monosomy of the long arm of chromosome 7, specifically 7q36 has been associated with holoprosencephaly, microcephaly, facial anomalies, growth and mental retardation and sacral agenesis. Objective- To investigate a 7 yr old girl with microcephaly, moderate hearing problems, partial sacral agenesis and developmental delay and compare the results with other cases of 7q terminal deletion. Materials and Methods- Blood from the child was analyzed cytogenetically by GTG banding in low resolution and then in high resolution. Fluorescent In Situ Hybridization (FISH) using subtelomeric probes and whole chromosome painting probes was performed followed by microsatellite analysis using 16 markers specific for chromosome 7. Result- The child was carrying a de-novo del(7)(q36.3) on the maternally derived chromosome. The deletion was found to be located between the markers D7S550 and D7S2465. It was also observed that out of the two main clinical manifestations associated with terminal 7q deletion, holoprosencephaly and sacral agenesis, the child carried only partial sacral agenesis. Conclusion- Since there is a wide spectrum of phenotypes for the terminal deletions and rearrangements on the long arm of chromosome 7, such cases highlights the significance and role of position effect. Perspectives- We are in the process of cytogenetic investigation of the childs skin cells and buccal smear to rule out the possibility of mosaicism. In order to explain her genotype-phenotype relationship it also becomes essential to look for the possibility of position effect. A microarray analysis is being performed to determine the genes lost and whether this deletion is a simple terminal one or if other cryptic anomalies are associated with it.

An Integrated Informatics Pipeline in support of multiplatform, large-scale genotyping. *G. Grant, J. Carey, P. Lin, E. Winchester, B. Bugalter, B. Handsaker, A. Wysoker, M. Nizzari, D. Mirel, A. Crenshaw, S. Gabriel* Broad Institute of MIT and Harvard, Cambridge, MA.

Genotyping studies today require data to be generated at a range of scales (of both SNPs and samples) and across a variety of platforms. Ideally, genotype data generated on one platform can be easily integrated with that generated on a complementary platform - for example whole genome genotyping data in a given cohort and targeted genotyping performed in follow up in the same cohort. We have developed a robust informatics pipeline to support and integrate whole genome platforms (Affymetrix and Illumina) as well as targeted genotyping (Sequenom and Illumina). At the core of the genotyping platform is the 'experiment packet' an object that allows all relevant aspects of a chip's progress through the lab and informatics workflow to be tracked. These aspects include workflow steps, reagent usage, allocation of resources and user-entered comments. Wherever possible, we have tried to use common steps (i.e. QC and archiving), but in some cases, the nature of the different products require different workflows. Affymetrix-specific steps include submission to GCOS, reading scan data from the scanner, calculation of QC metrics, and running of the Birdseed genotype calling program (developed at the Broad). Illumina-specific steps include supervision of scanning, running of Autocall to generate GTC files from the raw data, parsing of the GTC files to generate call rates and other metrics. Shared steps include several end-stage workflow steps. For instance, once the genotypes files for the chips are generated in their native format (Affymetrix Birdseed and Illumina GTC) the files are parsed into a compact generic binary genotype format (BGT). This file is then stored on a managed file system. Using a software API, the contents of these BGT files can be flexibly interpreted in order to run QC checks (including fingerprint and HapMap concordance verification). In conjunction with mapping information on the chips itself, the BGT files can used to serve up genotypes in a variety of formats for users of the pipeline. We present workflow steps, diagrams, and database schema.

Mutations in *TRIP11* cause neonatal lethal skeletal dysplasias in humans and mice. *P. Smits[1], A. Bolton[2], V. Funari[3], L. Lei[4], M. Hong[1], B. Merriman[3], S. Nelson[3], D. Krakow[3], D. H. Cohn[3], T. Kirchhausen[4], M. L. Warman[1,4,5], D. R. Beier[2,4]* 1) Children's Hospital Boston, Boston, MA; 2) Brigham and Women's Hospital, Boston, MA; 3) Cedars-Sinai Medical Center, Los Angeles, CA; 4) Harvard Medical School, Boston, MA; 5) Howard Hughes Medical Institute, Boston, MA.

Diverse biologic roles for thyroid hormone interacting protein 11 (TRIP11) have been suggested based upon *in vitro* and *ex vivo* biochemical and cell biologic studies. As part of an ENU-induced mutagenesis screen we produced a mouse line segregating an autosomal recessive perinatal lethal skeletal dysplasia. In addition to having short limbs and small thoraces, the newborn mutant mice failed to ossify their vertebral bodies. Chondrocyte proliferation and terminal maturation were abnormal and mutant chondrocytes had markedly elevated rates of apoptosis. At the electron microscopic level, mutant chondrocytes and osteoblasts had swollen endoplasmic reticulum (ER) and a misshapen Golgi apparatus. We identified a nonsense mutation in *Trip11* as the likely cause of the phenotype. The occurrence of dilated ER and misshapen Golgi in the mutant mice is consistent with a previously suggested role for TRIP11 in the Golgi, which was deduced from knock down experiments using RNAi in cultured cell lines. Despite ubiquitous expression of TRIP11, we did not observe ultrastructural evidence of ER swelling in other cells from the mutant mice, including keratinocytes, dermal fibroblasts, myocytes, and pericytes, although most lacked normal appearing Golgi stacks. The human disorder Achondrogenesis type 1A (Houston-Harris type) is characterized by neonatal lethality due to thoracic insufficiency, short-limbed dwarfism, absent vertebral body ossification, and chondrocytes containing swollen ER. DNA from an Achondrogenesis 1A proband, with consanguineous parents, was homozygous for SNPs across the *TRIP11* interval on chromosome 14 and for a 4 bp deletion within the *TRIP11* coding sequence that would frameshift and truncate the protein. These studies illustrate the utility of phenotype-driven genetic studies in model systems for the investigation of human disease and the importance of TRIP11 for skeletal growth in mammals.

Returning Copy Number Variant Findings to Research Participants: A Framework for Understanding Ethical and Clinical Implications. *H. Tabor, M. Cho, The Interdisciplinary Workshop on Returning CNV Results to Research Participants* Ctr Biomedical Ethics, Stanford Univ, Palo Alto, CA.

Rapidly-moving technological changes have allowed for the detection of copy number variants (CNVs), both de novo and inherited, across the entire genome and across hundreds and thousands of samples. Genetic research studies on complex traits are utilizing these new technologies to determine whether CNVs are associated with risk and to infer whether they might play important etiological roles in disease. As increasing numbers of research studies incorporate CNV analysis, many researchers are faced with dilemmas about the clinical implications of CNV findings and determining whether and how to return CNV results to research participants. In part, these concerns are rooted in long-standing controversies surrounding the return of individual genetic results, including debates over ownership of genetic information, interpretation of research results, and obligations of researchers to provide ancillary care, as well as duties emanating from the relationships between researchers and the research participants/disease groups whom they study. In part, however, CNV results present new challenges to researchers because of the impression that they may be more likely to be causal than other kinds of genetic variants. How should rare findings be interpreted, against the backdrop of copy number variation across the genome and populations? How do research participants, those with disease diagnoses, those at risk of disease, and those who are part of healthy normal populations, respond to and interpret CNV findings? An interdisciplinary group of experts met to identify the ethical and clinical challenges posed and develop a framework for deciding whether and how to return copy number variant results to research participants. The group included experts in CNV research, clinical genetics, genetics counseling, bioethics, social science, pediatrics and law. We will present the core challenges we identified and our work on developing a framework for researchers to use in determining whether and how to return CNV results.

The Effects of *ABCB1* Polymorphisms in Perinatal Depression. *A. K. Smith[1], D. J. Newport[2], C. L. DeVane[3], E. Zach[2], E. B. Binder[2], Z. N. Stowe[2], J. F. Cubells[1,2]* 1) Dept Human Genetics, Emory Univ, Atlanta, GA; 2) Dept Psychiatry & Behavioral Sciences, Emory Univ, Atlanta, GA; 3) Dept of Psychiatry & Behavioral Sciences, Medical Univ of South Carolina, Charleston, SC.

High levels of maternal depressive symptoms have been widely associated with poor infant outcome and provide compelling evidence to continue antidepressant treatment throughout pregnancy. *ABCB1* encodes P-glycoprotein (P-gp), which is integral in controlling the passage of substances at the blood-brain barrier and between maternal and fetal circulation via the placenta. Emorys Womens Mental Health Program performs longitudinal studies of women with depression throughout the perinatal period. For this study, we recruited 186 women in their 3rd trimester undergoing pharmacotherapy with antidepressants. We then examined the association between maternal polymorphisms in *ABCB1* and the maximum 3rd trimester score on the clinician-administered Hamilton Rating Scale for Depression (HRSD) using linear regression. Associations were observed with 3 exonic SNPs: rs1128503, rs2032582 and rs1045642 ($p < 0.0001$-0.002). Since these SNPs are in linkage disequilibrium, Haplo.Stat was used to examine the association between common haplotypes (>5%) and maximum HRSD score in the 3rd trimester. The G-G-C haplotype (53.9%) was associated with higher maternal depression ($p=6e-05$) while the A-T-C haplotype (28.6%) was associated with lower depression ($p=2e-05$). At delivery, cord blood was collected from the neonates for DNA extraction and to measure the levels of antidepressants in fetal circulation (N=78). Since placental P-gp is of fetal origin, we examined the association between neonatal genotypes and birth weight using a linear regression model that accounted for the level of antidepressant in the cord blood and its relative affinity for the P-gp transporter. Associations between the neonatal genotypes in 3 SNPs (rs2032582, rs2032583 and rs2235040) and high birth weight were observed ($p=0.0021$-0.049). The G allele of rs2032582 was associated with both higher maternal depression and higher neonatal birth weight, which has been associated with poor infant outcome. Though rs2032582 is a silent polymorphism, recent studies suggest that it can alter P-gp conformation and protein activity/substrate specificity. The role of *ABCB1* polymorphisms in antidepressant response, particularly during pregnancy, warrants further investigation. Our results suggest that neonates whose mothers have alleles that influence response to antidepressants may be at higher risk due to both increased maternal depression and antidepressant exposure, both of which may contribute to infant outcome.

A first look at the X chromosome in autism spectrum disorder through GWAS eyes. *E. R. Martin[1], R.-H. Chung[1], D. Q. Ma[1], J. Jaworski[1], J. R. Gilbert[1], D. Hedges[1], R. Abramson[2], H. Wright[2], J. Haines[3], M. L. Cuccaro[1], M. A. Pericak-Vance[1]* 1) Dept Human Genetics, University of Miami, Miami, FL; 2) School of Medicine, University of South Carolina, Columbia, SC; 3) Center for Human Genetics Research, Vanderbilt University, Nashville, TN.

The autism spectrum disorders (ASD) are a group of a complex neurodevelopmental disorders with a strong genetic component. The skewed prevalence toward males compared to females and evidence of linkage to the X chromosome in some studies suggests the presence of X-linked susceptibility genes for ASD. We have conducted the first analysis of GWAS data on the X chromosome in ASD families. We analyzed 1497 samples from 488 nuclear families in our Collaborative Autism Project (CAP) database, genotyped using the Illumina Human 1M beadchip. Results were validated in a second independent dataset of 3304 samples from 630 autism families from AGRE 550K SNP dataset. Markers were analyzed using the XAPL, a family-based test of association, in the overall datasets and sex-stratified datasets. Analysis of the overall CAP dataset identified strong association (p0.0001) for several markers in a region from 108.07-108.34 Mb. The AGRE dataset validated these results with peak association in the region at 108.73Mb (p0.0005). Two other markers around 143.88Mb and 144.70 Mb with p0.001 in the CAP dataset were in the region containing SLITRK2 and SLITRK4. This region was also validated with several significant markers in the AGRE dataset, with the most significant (p0.0001) at 143.95Mb. In addition to these findings the CAP dataset showed association (p0.0001) at markers in MAOB around 43.6Mb. Of note in the overall AGRE dataset, 3 SNPs with p0.0001 were in the dystrophin gene (DMD). Of the 22 SNPs with p0.001, a total of 13 were in DMD and 3 in the SLITRK2-4 region. This first GWAS analysis of the X chromosome has indentified and validated two regions, and highlights other putative candidate genes for ASD.

Detecting interacting quantitative traits loci using variance heterogeneity. *A. Skol[1], G. Du[2]* 1) Dept of Medicine, Section of Genetic Med, Univ of Chicago, Chicago, IL; 2) Dept of Statistics, Purdue Univ, Indianapolis, IN.

Genome-wide and candidate gene association studies generally identify disease variants or related quantitative traits using single marker tests. Tests of interaction have been relegated to secondary analyses due to the low power to detect such effects. We present a method to improve the power of interaction tests for quantitative trait loci by restricting tests to those markers that demonstrate unequal variance within each genotype class. Such heteroscedasticity is a natural result of the phenotypes within each genotype being a mixture of three distributions centered on different means (one for each genotype of the interacting locus). Standard tests of interactions are then performed between all pairs of loci showing unequal variances. The detection of heteroscedasticity may also be due to a gene by environment interaction; thus in addition to being an initial screen for interacting loci, our approach may be used as a general test for cryptic interaction, be it genetic, epigenetic, or environmental.

We examined our methods power to detect interacting variants in 3 two-locus interaction models. One with no marginal effects, another with one locus that has a marginal effect and a second that does not, and a third where both markers have marginal effects. We also compare our approach to one that selects markers to test for interaction based on nominally significant marginal effects. We find that when performing a typical candidate gene study in 2000 subjects, we have power of 50% to detect variants that account for > 2% of the phenotypic variability. We would have almost no power to identify such a locus when using marginal effects for initial screening when the model had no marginal effects. In order to have experiment-wise significance to detect unequal variances, such variants would need to account for nearly 10% of the phenotypic variability.

We are now exploring combining marginal effects and heteroscedasticity jointly to select markers for further testing for interaction effects.

Variation of collagen fibrils size by electron microscopy in patients with connective tissue disorders. *D. Babovic-Vuksanovic[1], E. Mihci[1], S. Jenkins[2], N. Lindor[1], J. Tarara[3], J. Parisi[4]* 1) Mayo College of Medicine, Department of Medical Genetics, Rochester, Minnesota; 2) Mayo College of Medicine, Division of Biostatistics, Rochester, Minnesota; 3) Mayo College of Medicine, Biochemistry and Molecular Biology, Rochester, Minnesota; 4) Mayo College of Medicine, Department of Pathology and Laboratory Medicine, Rochester, Minnesota.

Connective tissue dysplasias (CTD) are a clinically and genetically heterogeneous conditions, and for many affected individuals, diagnostic testing is not informative. CTDs are suspected based upon clinical findings of variable combinations and degrees of skin hyperelasticity, joint laxity, tissue fragility and vascular involvement. Ultrastructural studies of collagen fibrils of skin biopsies from patients with CTDs are not diagnostic, but may show heterogenity in collagen fibril diameter and shape. To correlate ultrastuctural findings with the clinical findings in individuals with suspected CTDs, we retrospectively evaluated EM findings in 14 patients, 9 of whom had clinical signs of CTD and 5 without findings suggestive of CTD, but who had skin biopsy for other diagnostic purposes (control group). For each patient, samples of 400-600 individual collagen fibrils (3-5 sets per individual) were examined. The minimum and maximum diameters were measured using semi-automated methods. The coefficient of variation (×100) of fibril diameter was calculated separately for each individual. Variability scores were compared between the groups using linear regression with generalized estimating equations to adjust for within-person correlation. Significantly more variability was found in the group of patients with clinical signs of CTD as compared to the control group ($p=0.004$). Our results suggest that the variation of fibriles size can be an important diagnostic tool in evaluation of patients with CTD. Further research on a larger group is needed to verify this difference.

A full mutation spectrum in Hirschsprung disease: a copy number analysis. *B. Q. Doan[1], C. Stewart[2], C. Kashuk[1], S. M. Arnold[1], A. Chakravarti[1], International HSCR Consortium* 1) IGM, JHMI, Baltimore, MD; 2) Bioinformatics, Boston College, Boston MA.

Multiple genes with rare and common risk variants and gene dosage sensitivity have been identified in Hirschsprung disease (HSCR). To better understand effects of variable gene dosage, we performed a CNV analysis on 233 trios from the International HSCR Consortium. For cost effectiveness, trios were run on either Affymetrix 500K NSP or STY chips. To identify CNVs, we applied two copy number analysis tools, Italics and STEPS, which use different aCGH segmentation algorithms. The 270 HAPMAP samples were also analyzed for an expected level of variation. Using Italics, we identified a total of ~5000 CNVs (72bp-110Mb) in HSCR, with an average length per individual of 500kb/32SNPs (NSP) or 270kb/22SNPs (STY). When affected offspring were analyzed separately, the average length of CNVs detected increased 80kb/5SNPs. Multiple regions were identified that warrant further investigation. A 1.4Mb de novo deletion on 6q25.1 was the most statistically significant variant, although present in only one affected offspring. Corresponding genotype data revealed high proportions of missing data, homozygous calls, and Mendelian errors supporting the presence of a true deletion. Examining all CNVs within this region, five more individuals with 1.3kb-62.9kb deletions were identified, as compared to only one 33kb deletion in HAPMAP. This suggests that although rare, the 6q25.1 region is potentially associated with HSCR. Of great interest is the identification of 665bp-112kb deletions (de novo and segregating) in 29 individuals on 9q31, which resides in the middle of a known linkage peak shown to be a genetic modifier. To understand their role, we are reassessing the CNVs in context of rare and common disease variants identified in our resequencing and genome wide association analysis from the same study population. To minimize follow-up of false positives, we are also developing statistical tests to incorporate copy number data with Mendelian error, homozygosity, missing genotype data rates, and prior genetic evidence to filter the numerous variants identified for those most biologically meaningful.

MicroRNA analysis of formalin fixed paraffin-embedded breast cancer tissues with different stages. *G. Xiao[1], Y. Zhao[1], C. Deng[2], H. Zhang[1], J. Xiao[1], R. Recker[1], Z. Gatalica[2], E. Silva[3]* 1) Genomics & Functional Proteomics Laboratories, Dept Med, Creighton Univ, Omaha, NE; 2) Dept Pathol, Creighton Univ, Omaha, NE; 3) Dept Surgery, Creighton Univ, Omaha, NE.

INTRODUCTION: Breast cancer still has the highest incidence among women in the United States. Our understanding of its molecular and cellular mechanisms is limited. Recently, microRNAs have gained favorable status as upstream regulators of breast cancer progression since then it can posttranscriptionally regulate sets of genes. It is now estimated that there are about 1000 miRNAs in the human genome, but only about 300 miRNAs have been identified in humans now. Much of miRNAs and their roles in cancer formation still await discovery. METHODS: Formalin fixed paraffin-embedded (FFPE) breast tissues from different stages of the cancer were de-waxed before performing total RNA extraction. Tissues, from normal breast, in-situ ductal carcinoma (DCIS), and invasive ductal carcinoma (IDC, stage II), with 15 subjects in each group were analyzed. The total RNA were extracted with acid-phenol: chloroform. MicroRNAs were isolated from total RNA. MicroRNA microarray profiling was performed using LC Sciences technology (LC Sciences, LLC). The Bioconductor implementation of Limma was used to analyze the data. RESULTS: MicroRNA profiling experiments have been performed from 45 FFPE breast tissues of breast cancer subjects. By comparing the endogenous miRNA level in normal to that in DCIS, we found that numbers of miRNAs (e.g. mir-21) were significantly induced, while some of miRNAs (e.g. mir-205) were suppressed, in DCIS stage. In subjects with IDC stage, we observed some up-regulated miRNA species (e.g. mir-21) and more down-regulated miRNA species (e.g. mir-126), indicating that those miRNAs could be important candidate mediators that regulate progressive process of the breast cancer. These findings were confirmed by real time microRNA RT-PCR using breast cancer patient samples. Further functional studies are still under investigation.

Developing and Validating a Breast Cancer Risk Estimator Based on Genetic Polymorphisms and Personal Factors. *J. J. Mulvihill[1], E. R. Jupe[2], N. S. Knowlton[3], L. P. Zhao[4], S. Manjeshwar[2], T. W. Pugh[2], D. C. DeFreese[2], B. A. Gramling[2], C. D. Shimasaki[2]* 1) Department of Pediatrics, OU Medical Center, Children's Hospital, Oklahoma City, OK; 2) InterGenetics Incorporated, Oklahoma City, OK; 3) NSK Statistical Solutions LLC, Choctaw, Oklahoma; 4) Division of Public Health Sciences, Fred Hutchinson Cancer Research Center.

BACKGROUND. Accurately estimating individualized risk of developing breast cancer is useful for early detection and cancer prevention. We hypothesized that combining genetic polymorphisms with clinical and demographic variables could improve estimates of individual breast cancer risk. METHODS. DNAs were genotyped for 117 common functional single nucleotide polymorphisms (SNPs) in candidate genes likely to influence human breast carcinogenesis. They were combined with clinical risk information collected in a large case-control association study from six regions of the United States to develop and validate a novel multifactorial risk estimator (MFRE) for breast cancer. The model-building set consisted of 5022 Caucasian women (1671 breast cancer cases and 3351 cancer-free controls). Two independent sample sets were used to validate MFRE performance: one consisted of 1193 Caucasian women (400 cases and 793 controls) and the other consisted of 581 African American women (164 cases and 417 controls). RESULTS. Application of multivariate logistic regression to the model-building set resulted in a MFRE employing 22 SNPs in 19 genes together with the Gail model risk factors. In the model-building and two validation sets, the MFRE exhibited a 1.7 to 2.2-fold improvement compared to the Gail model by assigning elevated risk estimates more accurately to breast cancer cases than to controls. In each validation group, the performance of the MFRE met or exceeded that of the model-building set. CONCLUSIONS. For the first time genetic information and epidemiological factors have been combined into a single model resulting in improved clinical utility for estimating age-stratified individual breast cancer risk.

Genetics teaching through science fiction: stories of students, appropriating concepts. *A. Ordonez, F. Suarez*
Instituto de Genetica Humana, Pontificia Univ Javeriana, Bogota, DC, Colombia.

The basic concepts of genetics involving the use of complex terminology, as the concepts of transcription, translation, genes regulation, biochemistry, phenotype, genotype and the study of the metabolic pathways have been of inadequate acceptance by the medical students during their early years of training. Similarly evaluating the courses which taught genetics, at medicines schools, has been traditionally based on multiple choice tests, a kind of evaluation that has showed that is not so reliable if the student understood or not the concepts taught. It was presented to medical students who enrolled the second year a new proposal for learning the basics of genetics. Using the concepts developed in the classes, the students were asked to create a fictitious medical history that included the learned concepts but showing their learning through stories of science fiction. The minimum conditions for developing the medical history of science fiction were: correlation phenotype-genotype, explanation of the mutation or polymorphisms, pathophysiology, possible treatments and diagnostic aspects. The students presented a total of 23 medical records where the fictional characters were properly representing the concepts developed at the classroom; students also sustained their ideas in articles of indexed journals to demonstrate the plausibility of certain molecular biology techniques that could eventually give sustenance to the fiction developed by the student. The advantages and future applications of this proposal are discussed.

What Does it Really Take to Re-Sequence a Human? *S. F. Nelson, N. Homer, B. O'connor, D. Skvortsov, H. Lee, Z. Chen, B. Merriman* Dept Human Genetics, UCLA Medical Ctr, Los Angeles, CA.

Rapid advances in massively parallel sequencing technology are bringing us to the era where whole genome re-sequencing will be an important component of biomedical research. This raises the practical question of what it really means to re-sequence a specific individual. Indeed, the only two instances that have been put forth so far, the published genomes of J. Craig Venter and James Watson, actually have rather low coverage and an uncertain level of completeness and accuracy. While these are extremely valuable first efforts, it remains unclear what can be expected from the new technologies, given their limited read length and read accuracy, and the limitations imposed by the large amounts of high-copy number, low-complexity sequence in the real human genome, as well as the degree of variation that exists in the population. To provide a rational framework for planning to re-sequence an individual with high confidence, we instead use large scale simulations to map out the relations between these parameters. Specifically, starting with the Human Genome Project reference as a model human genome, and adding variants drawn from standard variant databases to create a synthetic diploid genome, we then create large-scale (~million to ~billion) read sets drawn from this genome and study the relation between coverage, read length, read accuracy, and end-pairing strategy, and the fraction of the genome that is accurately resequenced. We also estimate the fractions of the genome that cannot be re-sequenced at all, due to their highly repetitive structure, or those that can only be semi-uniquely resequenced, due to large scale repeats within the genome. This systematic survey allows us to set reasonable expectations for what can be accomplished now, to select optimal strategies for doing so, and also allows us to set near-term performance goals that are desirable milestones for the new sequencing technologies.

No Evidence of Differential Gene Expression in Chronic Fatigue Syndrome. *A. Byrnes[1], A. Jacks[2], B. Evangard[2], K. Dahlman-Wright[3], F. Wright[1], N. Pedersen[2], P. Sullivan[2,4]* 1) Dept of Biostatistics, Univ of North Carolina, Chapel Hill, NC; 2) Dept of Medical Epid & Biostatistics, Karolinska Institutet, Stockholm, Sweden; 3) Novum, Karolinska Institutet, Stockholm, Sweden; 4) Dept of Genetics, Univ of North Carolina, Chapel Hill, NC.

Background. Chronic fatigue syndrome (CFS) is defined by idiopathic and debilitating fatigue, and may be caused by immune dysregulation. The goal of this study was to identify transcriptomic biomarkers for CFS by studying MZ twins discordant for CFS. Subjects. We screened 61,000 Swedish twins to identify 44 pairs of proven MZ twins who were rigorously discordant - one twin was currently affected with CFS or the closely related idiopathic chronic fatigue (ICF) and the co-twin had never had chronic fatigue. All subjects were clinically evaluated by experts in CFS on the same day and time. A venous blood sample was collected into RNeasy and Affymetrix HG-U133 Plus 2.0 microarrays (54,675 transcripts) were run per protocol. Intensity values were normalized using the RMA algorithm and analyzed with modified paired t-tests using SAM that contrasted an affected twin with the unaffected co-twin. Results. The primary analysis found that no transcripts showed significantly different expression after correction for multiple comparisons. Secondary analyses consisted of the subset of 37 female twin pairs (i.e., dropping male pairs) and of 28 female twin pairs where the affected twin met the more rigorous criteria for CFS (i.e., dropping pairs where the affected twin had ICF). No transcript showed significant differential expression in either secondary analysis. Conclusions. These results suggest that CFS is not associated with significant differential gene expression in PBLs detectable with this technology. Moreover, prior studies that found differential gene expression in CFS may have been an artifact of inadequate case-control matching. Alternatively, it is possible that there is differential expression, but that these transcripts are expressed at low levels or undetectable with this microarray.

Functionally annotated genomic sequences are enriched for expression trait polymorphisms. *M. Xu[1,2], A. Murphy[1], R. Lazarus[1], S. T. Weiss[1], B. A. Raby[1]* 1) Channing Lab, Harvard Medical School, Boston, MA; 2) Bioinformatics Program, Boston University, Boston, MA.

In contrast to coding variants, distinguishing functional non-coding regulatory single nucleotide polymorphisms (SNPs) from among the millions of known non-genic variants is challenging. Currently available prediction algorithms rely on position weight matrix approaches and sequence conservation. The ever-increasing availability of functional sequence annotation through efforts like the ENCODE project may enable development of more accurate prediction algorithms. However, it is unclear which of the many forms of sequence annotation being generated is most useful for identifying expression trait SNPs. Using results from three genome-wide expression trait mapping studies (Dixon, Stranger, and a third unpublished dataset) and 16 annotation tracks available through the UCSC Genome Browser, we assessed whether functional DNA sequence was enriched for cis-acting expression trait SNPs. In all three datasets, we found significant enrichment for cis-acting expression trait SNPs for most functional tracks. Greatest enrichment (>3 fold increase in all three datasets, p<10-16) was noted for SNP mapping to CpG islands, RNApolII sites, DNase hypersensitivity sites, histone modification sites, and microRNA target sites. In contrast, enrichment was less impressive and was inconsistent when using sequence conservation data (i.e. regPotential 5x and 7x) or transcription factor binding site prediction tracks. These findings were independent of SNP distance from transcription start site. Notably, enrichment of expression trait SNP within functional sequence was greatest for those SNP demonstrating reproducible association with gene expression in two or more studies. These data suggest that available functional sequence annotation databases can be used for classification of SNP for genetic association studies. Funding: U01 HL065899, P01 HL083069, R01 HL086601, K08 HL74193.

AN INTRONIC *MEN1* MUTATION IS ASSOCIATED WITH PROLACTINOMA IN A YOUNG BOY, DECREASED PENETRANCE IN HIS LARGE FAMILY, AND VARIABLE EFFECTS ON MENIN mRNA AND PROTEIN. *L. Drori-Herishanu[1], A. Horvath[1], M. Nesterova[1], Y. Patronas[1], M. Lodish[1], N. Patronas[2], S. Agarwal[3], R. Salvatori[4], V. Mericq[5], CA. Stratakis[1]* 1) NICHD, NIH, Bethesda, MD; 2) DDR,NIH,Bethesda,MD; 3) NIDDK,NIH,Bethesda MD; 4) John Hopkins University,Baltimore,MD; 5) University of Chile,Casilla.Chile.

Prolactinomas are extraordinarily rare tumors in prepubertal children. Even in the absence of family history or other symptoms, a prolactinoma in a young child may be due to novel sequence variants of genes that are known to cause these tumors (menin, *PRKAR1A*, *AIP*). We report the case of a 7 year-old boy with a macroprolactinoma who was treated with cabergoline and the tumor receded. We studied him and his family for genetic causes of this tumor. A heterozygous substitution was identified (IVS3-7 c>a) in intron 3 of the *MEN1* gene. There were no mutations in the *PRKAR1A* and *AIP* genes and the defect was not present in an extended set of controls. However, normal individuals did express at low levels the mRNA predicted by lack of splicing of intron 3, incorporating an additional 210 base pairs into the mRNA, which leads to a prematurely terminated protein. The latter, however, was not found in protein lysates of the patients and controls; instead, what was found in the former (but not in the latter) was a longer menin variant which was only present in the cytosol. Menin protein levels were decreased in the carriers of the IVS3-7 c>a defect. We conclude that a novel MEN1 defect was found in a young boy with prolactinoma and his family. It is apparently associated with low penetrance of the disease. The IVS3-7 c>a defect is pathogenic because it leads to lower menin levels in the cells of these patients, but its effects may be mitigated by a variety of factors including changes in transcription and translation of the *MEN1* gene including the cytosolic presence of an unknown menin variant only in carriers.

Frequency of C3435T single nucleotide MDR1 genetic polymorphism in Native and Mestiza Mexican Populations. *L. SANDOVAL-RAMÍREZ[1,2], J. BECERRA-CONTRERAS[1,2], S. G. OROZCO-FLORES[1,2], E. R. OCHOA-MARTÍNEZ[1,2], J. M. OLIVA-ORTIZ[1,2], L. B. LÓPEZ-HERNÁNDEZ[1,2], M. CASAS-CASTAÑEDA[1,2], F. RIVAS[1,2]* 1) DIVISIÓN DE GENÉTICA, CIBO, IMSS, GUADALAJARA, JALISCO, MEXICO; 2) DOCTORADO EN GENÉTICA HUMANA, UNIVERSIDAD DE GUADALAJARA. GUADALAJARA, MEXICO.

BACKGROUND: P-glycoprotein is a membrane protein encoded by the MDR1 gene, which demonstrates functional polymorphism. The MDR1 single nucleotide polymorphism 3435C>T is associated with higher P-glycoprotein expression. OBTECTIVES: To determine allelic and genotype frequencies of C3435T single nucleotide MDR1 genetic polymorphism in Native and Mestizo Mexican populations. METHODS: 415 DNA samples of unrelated individuals were analyzed; 48 Huicholes (North of Jalisco and east of Nayarit), 48 Purepecha (Michoacan state), 60 Tarahumaras (Southeast of Chihuahua) and 215 Mestizos (Oaxaca-Guerrero coast, Chihuahua and Veracruz states). RESULTS: It was found a high percentage of heterozygous in Native and Mestizo populations. The distribution of Huichol population was homozygous for the wild-type allele (CC) was 27.08%, 50% were compound heterozygous with a mutant T-allele (TC), and 22.91% were homozygous for the mutant allele (TT). In Purepecha population the frequencies were: CC (25%), TC (45.83%9 and TT (29.16%), in Tarahumara population: CC (16.66%), TC (63.33%) and TT (20%) and in Mestizos: Oaxaca-Guerrero coast: CC: (27.38%), TC (46.42%) and TT (26.19%), Chihuahua state population: CC (22.22%), TC (44.44%) and TT (33.33%) and Veracruz state population: CC (22.09%), TC (43.02) and TT (34.88%). DISCUSSION: Allelic and genotype frequencies of 3435C>T changed meaningfully in populations with different origins, with a prevalence of the allele C in certain parts of the world (>70% in African populations), on the contrary in Caucasian and Asian populations, where this allele shows frequencies from 30% to 50%. CONCLUSIONS: We found a difference in the distribution of frequencies in the wild-type allele between a Germany population (reference population) and the Native populations (Tarahumara and Purepecha). On the other hand the percentage of heterozygous was high in the other populations (Mestizo and Huichol).

Submicroscopic tandem duplications - unraveling the mechanisms of formation. *P. Stankiewicz[1], K. Derwinska[1,2], Z. Xia[1], M. Nesteruk[1,2], B. Wisniowiecka-Kowalnik[1,2], M. Smyk[1,2], S. W. Cheung[1]* 1) Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX; 2) Dept of Medical Genetics, Institute of Mother and Child, Warsaw, Poland.

Cryptic chromosomal duplications have been long under-ascertained. Using a clinical targeted array CGH CMA-V6 (Agilent, 44K), we have identified seven potentially pathogenic microduplications in patients with normal karyotypes. Application of 385K and 2.1M microarrays (NimbleGen) and long-range PCR determined their tandem orientation in all cases and allowed rapid sequencing of their junction fragments. We have analyzed DNA sequence of 14 breakpoints of: a 280,796 bp duplication encompassing *KCNK16* (epilepsy), a 363,599 bp duplication harboring *PTCH1* (microcephaly), an intragenic 46,451 bp duplication in *DMD* (learning difficulties), a 120,772 bp duplication 7,326 bp 5 to *DAX1*, a 8,846,307 bp duplication in 1p32.3-p31.3 (dysmorphic features, failure to thrive, and autistic behavior), an intragenic 184,260 bp duplication in *NRXN1* (autism), and a 524,226 bp duplication involving *GALNT*. The duplication of exons 17 and 18 in *DMD* led to a 100 amino acids insertion in the dystrophin protein, and the duplication of exons 3-5 in *NRXN1* resulted in a premature protein truncation. Three chromosomal breakpoints mapped within LINEs, one within SINE/MIR and one within DNA/MER1 Charlie2a element. In eight other cases, five LINEs, two SINEs (*Alu* and MIR), and one DNA/MER1 MER5A1 element mapped less than 400 bp from the breakpoint. In two cases (*KCNK16* and *NRXN1*), we have identified TGAAAGTGT and ACCGGG insertions at the breakpoint junctions and microhomology (1-3 bp) between the breakpoints in four microduplications, indicating a nonhomologous end joining mechanism of their formation. In addition, we analyzed DNA sequence of 12 LCR-free breakpoint regions of 6 cryptic likely polymorphic copy-number gains identified using 2.1M microarrays (average resolution ~1.5 kb). We found LINE elements at eight and SINE elements at eight breakpoints. Our data support the results reported recently showing significant (~30%) proportion of retrotransposon elements at the CNV breakpoints.

Theoretical Investigation of the Polymorphism Pattern Since a Selective Sweep. *H. Chen[1], K. Chen[2]* 1) Department of Genetics, Harvard Medical School, Boston, MA.02115; 2) Department of Statistics, University of California, Davis, CA 95616.

The fixation of an advantageous allele in a population can affect the polymorphism pattern of the nearby neutral loci, which is called a selective sweep. This kind of data pattern can be informative for both detecting selection and inferring the selection intensity and the time since the sweep. Previous work either infers the selection intensity by assuming data is observed right after the selection, or by using simulations lack of theoretical foundations. We analytically investigate the decaying of the skewed pattern with time. The approximate sampling formula by Etheridge et al(2006) is adopted to model the effect of a sweep on a linked neutral locus. Using both the coalescent model and the diffusion equation, different aspects of the polymorphism pattern, including the frequency spectra of the segregating sites, the polymorphism heterozygosity and the number of alleles are derived as a function of the selection intensity and the time of the sweep. Based on our theoretical results, we draw conclusions on the decay of the skewed pattern. As one application of the results, we develop a likelihood method for jointly estimating the selection intensity and the time since the sweep. The method is applied to the real data FOXP2 to demonstrate its performance.

Clinical and biochemical characterization of a chromosome Xq28 microdeletion encompassing at least 9 genes including *ABCD1* and *SLC6A8*. *J. J. Shen[1], J. A. Bernstein[2]* 1) Dept of Medical Genetics & Metabolism, Childrens Hospital Central California, Madera, CA 93636; 2) Division of Medical Genetics, Dept of Pediatrics, Stanford University School of Medicine, Stanford, CA 94305.

The Xq28 genomic region contains several genes known to cause human disease, including *ABCD1* (X-linked adrenoleukodystrophy), *SLC6A8* (X-linked creatine transporter deficiency) and *L1CAM* (L1-spectrum disorders). Deletions affecting these genes individually, as well contiguous gene deletion syndromes involving this region [*e.g.*, CADDS (Contiguous *ABCD1*/*DXS1357E* Deletion Syndrome)] have been described. We report the clinical and biochemical characteristics of a patient with an Xq28 microdeletion encompassing at least 9 genes, including *ABCD1* and *SLC6A8*.

The patient was a former 33 week male infant whose clinical issues included failure to thrive, GI malabsorption, and cholestasis. On exam, he had craniofacial dysmorphisms, slender fingers and toes, and microphallus. Echocardiogram demonstrated two small VSDs, eye exam revealed anterior iris stroma hypoplasia, and liver biopsy showed evidence of cholestasis but no paucity of bile ducts. Head and abdominal ultrasounds as well as plain films of the spine were normal. Endocrinologic evaluation demonstrated normal pituitary function and isolated hypogonadism. Cystic fibrosis and Alagille syndrome were ruled out. Through oligonucleotide array-CGH, a microdeletion in Xq28 was identified. Based on the breakpoints, biochemical studies were performed. VLCFA analysis was consistent with a peroxisomal disorder, and studies of creatine metabolism suggested X-linked creatine transporter deficiency. He passed away at 4 months of age due to liver failure.

The clinical course and biochemical profile seen in this patient with an Xq28 microdeletion was similar to that described in CADDS, and he had further evidence of a defect in creatine transport, consistent with a missing *SLC6A8* gene. He also had hypogonadism, likely because additional genes were affected by the microdeletion. This patients phenotype expands our understanding of the clinical consequences of genomic disorders involving the Xq28 region.

Detection of copy number imbalances in lung adenocarcinoma is enhanced by laser capture microdissection. *A. C. Borczuk, O. Nahum, L. Herlitz, C. A. Powell, B. Levy* Department of Pathology, Columbia University, New York, NY.

Mixed subtype pulmonary adenocarcinomas frequently have an admixture of tumor cells, desmoplastic stroma and inflammation, as well as areas of residual alveolar walls in areas of lepidic growth. Sections that contain over 70% tumor are preferentially used for studies of DNA copy number imbalances. However, many tumor sections have far less representation of tumor cells (<< 70%) or they contain significant numbers of benign inflammatory cells. The net effect is a reduction in the sensitivity of CGH methodologies for detection of copy number gains and losses in the tumor specimen. Frozen sections of mixed subtype adenocarcinoma were either manually needle dissected or laser capture microdissected using a PALM Zeiss Microbeam System to obtain a minimum of 500 ng of genomic DNA for study on Affymetric 6.0 SNP microarrays. Laser capture microdissected material identified all alterations that were detected by needle microdissection, confirming that laser capture did not result in poor DNA quality. Laser capture microdissected tumor allowed for detection of increases and decreases in copy number in multiple chromosomal arms; overall, alterations in 19 chromosomal arms in one pair of samples and 11 chromosomal arms in the other pair were detected in the LCM samples only. The majority of these alterations comprised small deletions that would not be expected to be detected in a specimen of heterogenous cell populations. Laser capture microdissection of mixed subtype lung adenocarcinoma allows for detection of numerous regions of copy number gains and losses that would not be identified by whole section homogenates or manual needle dissection enrichment. This creates the opportunity to study copy number changes in early neoplasia and tumors that contain abundant admixed non-neoplastic stromal or inflammatory cells.

***Caenorhabditis elegans bbs* mutants have body size defects.** *C. A. Mok[1,3], E. Héon[1,2], M. Zhen[3]* 1) Program in Genetics and Genome Biology, Sick Kids Hospital, Toronto, ON, Canada; 2) Department of Ophthalmology and Vision Sciences, Sick Kids Hospital, Toronto, ON, Canada; 3) Mount Sinai Hospital Research Institute, Toronto, ON, Canada.

Bardet-Biedl syndrome (BBS) is an autosomal recessive, genetically heterogeneous, pleiotropic disorder. Cardinal features include photoreceptor degeneration, obesity, digit anomalies, kidney anomalies, cognitive impairment and hypogonadism. BBS genes include ciliary proteins related to the intraflagellar transport (IFT) system. *C. elegans bbs* mutants have defects to ciliary morphology and function (Blacque et al., 2004, Tan et al., 2007). Neurosensory defects in *C. elegans* have been associated with decreased body length (Fujiwara et al., 2002) and increased fat accumulation (Mak et al., 2006). The transforming growth factor beta (TGF-) superfamily of proteins encompasses those in a pathway that also controls body size (Patterson and Padgett, 2000). The vertebrate orthologs of this pathway form the bone-morphogenic proteins (BMPs) and are important to early embryonic development, patterning, and skeletal formation. We hypothesize that *C. elegans bbs* mutants will share specific phenotypes related to various ciliated-neurons. We examined *C. elegans bbs* mutants, and indeed, body length measurements showed a statistically significant loss of 15-20% in mean body length with no increase in body width. DAPI nuclei staining of *bbs* mutants indicate no gross defects in cell number. Analysis of TGF- mutants (*dbl-1* and *lon-2*) showed that *bbs* body size defects are not epistatic to this pathway. In conclusion, *bbs* body length defects are likely due to decreased cell volume in a pathway separate from that of the TGF- body length pathway. The non-cell autonomous control of body size through a subset of ciliated neurons identifies an interesting pathway for further study in *C. elegans* and later study of our human BBS cohorts.

SNP genotyping in large sample sets using a miniaturized PCR array platform. *S. Liu-Cordero[1], H. Ranu[2], E. Ortenberg[1], A. Bond[1], K. Munnelly[1], D. Hunter[2]* 1) BioTrove Inc., 12 Gill St., Suite 4000, Woburn, MA 01801; 2) Brigham and Woman's Hospital - Channing Laboratory 181 Longwood Ave Boston, MA 02115.

Robust genotyping technologies and the availability of the International HapMap Project data have made genome-wide association studies possible. The number significant results coming from these studies are rapidly increasing and have highlighted the next challenge of validating these results in larger sample sets with a smaller and more refined set of assays. In this study we test the utility of a high density and low-volume PCR array for mid-range genotyping applications. The array consists of 3072 holes that can be loaded with reagents to perform individual 33 nL reactions. The surfaces of the array are treated to have hydrophilic coatings on the interior of each hole and hydrophobic coatings on the exterior. This enables solutions to be accurately loaded in the holes via capillary action. The unique configuration of the holes in the array allowed for the interrogation of a large number of DNA samples against a large number of assays in a flexible, configurable format. We tested a number of assay configurations, including sets of 16, 32 and 64 SNPs within candidate regions. These assay sets were tested on sample populations from a variety of genetic epidemiological studies such as diabetes, skin, colon, and endometrial cancers. The data showed high quality genotype cluster data and high genotype call rates. This platform provided an efficient and robust solution for completing a large number of projects each consisting of thousands of samples.

Biological properties of a novel AML1 mutation in a family with Familial platelet Disorder/Acute Myeloid Leukemia Syndrome. *A. Sorrell[1], W. Wang (Co-author)[2], J. Weitzel[1], P. Parker[3], R. Bhatia[3]* 1) Clinical Cancer Gen, City Hope National Medical Center, Duarte, CA; 2) Div. of Population Sciences; 3) Div. of Hematology and Transplantation.

AML1/RUNX1 mutations (linked to 21q22.1-22.2) are associated with a rare form of Autosomal Dominant, familial thrombocytopenia, with a predisposition to adult-onset myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). At least 10 different causative AML1 gene mutations have been described in families with Familial platelet Disorder/Acute Myeloid Leukemia Syndrome (FPD/AML). Families carrying mutations that retain greater degrees of biological activity (e.g. deletions and frameshift mutations that act via haploinsufficiency) have a ~ 20% risk of MDS/AML as compared to a ~ 60% risk in families that carry mutations with little residual activity (e.g. dominant negative or biallelic nonsense mutations). The functional significance of AML1 mutations can provide valuable information needed for cancer risk assessment of FPD/AML families. We have identified a novel AML1 gene mutation that tracks with disease in an American family of Western/Northern European descent. Genomic DNA from our proband (37-year old with thrombocytopenia and eczema) and his mother (68-year old with MDS, thrombocytopenia, and eczema) were analyzed using PCR amplification and bidirectional sequencing of all 8 exons of the AML1 gene. They each carry one copy of a frameshift mutation (nc1413_1414 insGC) at the 3 end of exon 8, which is predicted to bypass the normal stop codon and produce a run-on RNA product. If stable, this run-on RNA product could be translated into a substantially extended protein product (p.Leu472fsX123). The biological characteristics and functional significance of this mutation are not known. The aims of this study are to 1) characterize the genotype-phenotype associations within this family and to 2) use immunoblotting and functional assays to determine the biological significance of this novel mutation. We are currently performing segregation analyses, further characterization of the phenotype, and confirmatory studies using hematopoietic samples from additional family members.

Confirmation of Dynapsin Association in Alzheimer Disease. *P. L. Whitehead[1], J. R. Gilbert[1], S. Züchner[1], G. Wang[1], C. Kroner[1], C. Lu[1], P. Bronson[2], P. Gallins[1], M. A. Slifer[1], D. D. Vance[1], S. M. Garvey[2], P. Xu[2], W. K. Scott[1], Y. Li[2], J. M. Vance[1], J. L. Haines[3], E. R. Martin[1], M. A. Pericak-Vance[1]* 1) Miami Inst Human Genetics, Univ Miami , Miami, FL; 2) Duke University Medical Center, Durham, NC; 3) Center for Human Genetics Research, Vanderbilt University Medical Center, Nashville, TN.

Unraveling the complex genetics underlying Alzheimer disease (AD) is critical for understanding this devastating condition. Previously, through linkage, and association analyses, we identified 3 key SNPs (rs12622006, rs10932212, and rs13432421) on the q arm of chromosome 2 that showed significant association with intermediate-onset familial AD (IOAD) (p=.002). IOAD encompasses AD families with a mean age-at-onset > 60 yrs that have at least one early-onset individual (< 60 years). Through the convergence of our statistical results and Serial Gene Expression Analysis(SAGE) we implicated LOC389072/dynapsin as a novel IOAD risk gene. Dynapsin is expressed in neurons of AD neuritic plaques and neurofibrillary tangles and interacts with the dynein-dynactin motor complex. In order to validate our previous significant association results,18 SNPs in and surrounding dynapsin were further interrogated in a second independent non-Hispanic Caucasian cases (N=74)/cognitively normal controls (N=108) IOAD dataset. Analysis of the new independent dataset of IOAD patients confirmed a risk effect (p=0.02) for the dynapsin SNPs and alleles. Joint analysis of the combined discovery and validation datasets further strengthened the risk effect (p=0.001), with the odds ratio for SNPs rs12622006, rs10932212, rs13432421 being OR 2.59, 95% Cl[1.4, 4.7], OR 2.68, 95% Cl[1.4,4.9], OR 2.81, 95% Cl[1.4, 5.2], respectively. Our results strongly support that variation in the region of the dynapsin gene contributes to the risk of familial AD.

***NF1* exon23a alternative splicing in neurofibromas and MPNSTs.** *R. Loda, H. Li, M. Wallace* Dept Molec Genetics/Microbiol, Univ Florida, Gainesville, FL.

Three well-characterized alternatively spliced exons have been identified in the *NF1* gene. Mutations in this gene cause neurofibromatosis 1 (NF1), a common autosomal dominant disorder in which individuals are predisposed to neurofibromas and other tumor types. Inclusion of alternately spliced exon23a inserts 21 amino acids in the GTPase activating protein (GAP)-related domain of the encoded protein, neurofibromin. This insertion reduces the proteins ability to down-regulate Ras, currently neurofibromins major known function in the cell. *NF1* mRNA containing exon23a (Type II mRNA) is present at equal or slightly greater levels than Type I mRNA (no exon23a) in the majority of adult tissues reported, and has been reported at aberrantly high levels in some non-NF1 related cancers. We have analyzed the relative levels of Type I/Type II mRNA in blood (non-NF1 patients), in primary dermal and plexiform tumors (NF1-derived), as well as neurofibroma and malignant peripheral nerve sheath tumor (MPNST) Schwann cell cultures. We found Type I transcript levels in blood to be greater than or equal to Type II. However, dermal and plexiform tumors and cultures had Type II mRNA as the predominant transcript, with some tumors having very little or no Type I transcript present. These samples were of interest based on a previous report of MPNSTs with high levels of Type II mRNA exhibiting RNA editing, leading to a premature stop codon. However, analysis of several of our tumors (including an MPNST) with little or no Type I transcript found no evidence of RNA editing. Of interest, an MPNST with no Type I transcript has a classic microdeletion of the *NF1* gene and flanking sequences as its germline mutation, but no somatic *NF1* mutation has been found in the entire open reading frame of the remaining allele (at the DNA or RNA level). This raises the question of whether the Type II transcript could be a hypomorph, which could be contributing to tumorigenesis by virtue of reduced Ras-GAP activity.

Promoter methylation patterns of ATM, ATR, BRCA1, BRCA2 and P53 as putative cancer risk modifiers in Jewish BRCA1/ BRCA2 mutation carriers. *E. Friedman[1], T. Kontorovich[1], Y. Cohen[2], U. Nir[3]* 1) Oncogenetics Unit, Inst Gen, Chaim Sheba Medical Ctr, Tel Hashomer, Israel; 2) Dept. of Gyneco-Oncology, Sheba Medical Center, Tel-Hashomer, Israel; 3) Mina and Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Israel.

Background: BRCA1/BRCA2 germline mutations substantially increase breast and ovarian cancer risk, yet penetrance is incomplete. Genetic and environmental factors putatively modify penetrance. We hypothesized that germline epigenetic gene silencing may also affect penetrance. To test this notion, we determined the methylation status of the promoter in several candidate modifier genes in Jewish BRCA1/BRCA2 mutation carriers Methods: Asymptomatic (n=41) and breast cancer affected (n=48) BRCA1/2 mutation carriers, breast cancer non-carriers (n=52), and healthy controls (n=89), were all genotyped. Real-time, methylation-specific PCR was used for detection and quantitation of promoter hypermethylation in the BRCA1, BRCA2, ATM, ATR and P53 genes. Results: Promoter hypermethylation was detected only for BRCA1, noted in 14 of 230 samples analyzed. There were no differences in the rate of BRCA1 promoter hypermethylation between the study groups: BRCA1/2 mutation carrier breast cancer patients: 6.25% methylated, BRCA1/2 mutation carrier asymptomatic: 7.31% methylated, sporadic breast cancer: 5.76% methylated and controls: 5.62% methylated. Conclusions: Although methylation of the promoter region of BRCA 1, BRCA2, ATM, ATR and P53 genes does not substantially contribute to phenotypic expression of mutant BRCA1/BRCA2 alleles, germline promoter hypermethylation in the BRCA1 gene can be detected in about 5% of the female population, regardless of the BRCA1/2 status. The significance of this observation is yet to be determined.

The association of AVPR1A with addiction is mediated by spousal relationship quality. *B. S. Maher[1], S. J. Latendresse[1], M. Kang[1], B. Devlin[2], R. E. Ferrell[2], G. P. Kirillova[2], L. Kirisci[2], R. E. Tarter[2], K. S. Kendler[1], M. M. Vanyukov[2]* 1) VIPBG,Virginia Commonwealth Univ, Richmond, VA; 2) Univ Pittsburgh, Pittsburgh, PA.

The risk for addictions is largely non-specific to particular drugs and is highly genetically correlated with premorbid traits reflecting regulation of social behavior/antisociality. Involvement with illicit drugs by definition requires violation of social norms. An important role in social behavior has been attributed to the neuropeptide arginine-vasopressin (AVP), shown to be involved in mate and social relations and aggression. We hypothesized that variation in a vasopressin receptor gene, AVPR1A, influences the risk for addiction to illicit drugs, and that this association is mediated by the characteristics of mate/spousal relations. In a sample of 398 individuals diagnosed with a drug use disorder and 359 controls we performed association analysis between 1536 SNPs in 106 candidate genes and a dichotomous diagnosis of drug use disorder (DUD). The quality of mate relations was evaluated by the Dyadic Relationship Scale of the Family Assessment Measure (FAM). Two SNPs in the AVPR1A gene demonstrated the strongest association with DUD risk, rs1587097 (p=0.0003, OR=2.02 [1.37-3.00]) and rs10784339 (p=0.0008, OR = 1.72 [1.25-2.36]); gene-wise permuted p=3x10-5. The FAM score was significantly associated with DUD in males (p<0.0001), but not females (p=0.09). The AVPR1A SNPs were significantly associated with FAM factor score in males (p=0.007), but not females (p=0.74). The mediation hypothesis was further tested using structural equation modeling. All direct paths - AVPR1A and FAM, FAM and DUD, and AVPR1a and DUD - were significant (p=0.006, p<0.001, and p=0.006, respectively). Most importantly, the indirect (mediated) path was also significant (p=0.013), supporting mediation of the association between AVPR1A and addiction risk by the FAM. (R01DA011922, K02DA018701, P50DA005605, K02DA017822, and R01DA01957).

Distinct patterns of gene expression in complex disease genes. *D. Torgerson, C. Ober* Department of Human Genetics, University of Chicago, Chicago, IL.

Identifying genes that contribute to human disease is an important step towards understanding disease etiology, and can facilitate the development of diagnostic tools, preventive medicine, and novel treatments. Previous studies have suggested that evolutionary signatures of natural selection can facilitate the identification of disease genes, as they tend to show stronger signatures of positive and negative selection. We identified complex disease genes from the Genetic Association Database, and Mendelian dominant and recessive disease genes from OMIM in order to identify additional characteristics of disease genes that could be used in the same way. Using the results from a genomic scan for natural selection, we find that complex disease genes have only moderately higher probabilities of negative selection than non-disease genes (Mann-Whitney U-test, p=0.04). However, a more striking pattern was observed in gene expression profiles from 71 normal human tissues using the geneAtlas2 data. We find that complex and Mendelian disease genes have a significantly higher index of tissue specificity () than other genes (MWU-test, p0.001), suggesting that disease genes are more tissue specific in their expression. We also find that mean expression level is significantly higher in disease genes (MWU-test, p0.001), despite their being expressed in fewer tissues. This results from disease genes having a significantly higher absolute level of gene expression in their tissue of maximum expression (MWU-test, p0.001), suggesting that disease genes are more likely to be highly expressed, tissue-specific genes. Our results suggest that both complex and Mendelian disease genes exhibit distinct patterns of gene expression, and identify an additional biological characteristic that may facilitate the identification of novel disease genes.

Evaluation of DNA damage in bone marrow of acute lymphoblastic leukemia patients by comet assay and blood reference group. *L. Bobadilla-Morales[1,2], C. E. Monterrubio-Ledezma[1], R. Silva-Cruz[1], M. A. Orozco-Martín[1], A. L. Fletes-Rayas[1], A. R. Hernández-Robles[1], A. Márquez-Mora[1], F. Sánchez-Zubieta[2], V. Soto-Chávez[2], A. Corona-Rivera[1,2]* 1) Universidad de Guadalajara, Departamento de Biología Molecular y Genómica, Instituto de Genética Humana "Dr. Enrique Corona Rivera", Laboratorio de Citogenética Genotoxicidad y Biomonitoreo; 2) Unidad de Citogenética, Servicio de Hemato Oncología Pediátrica, Hospital Civil Dr. Juan I. Menchaca.

Cancer consists of uncontrolled cellular clonal proliferation and genetic instability. The most frequent cancer in children is leukemia. It consists of an irregular clonal expansion of immature lymphoid or myeloid stem cells. The X-ray treatment of this disease shows mutagenic effects including breaking in the DNA. The comet assay is a standard method to evaluate damage to DNA. Since there might be differences in the response of ALL cells to radiation exposition, we want to test DNA damage in ALL patients before treatment onset, measured by comet assay. We considered bone marrow from 10 patients. We included also a reference group composed of 10 peripheral blood samples from 10 healthy controls matched by age and sex (ages, between 3 ms to 16 yrs). Comet assay was performed in each sample as follows: basal, 0, 30 and 60 min. of culture after exposition to 2Gy of radiation as well as 30 and 60 min. without radiation exposition. 50 cells were analyzed per condition, measuring tail, nucleus and total length as well as qualitative measures as Urbina et al. 2006. There were no statistical differences between patient cell tested conditions, nor reference cell conditions. DNA damage was increased in patient cell conditions versus reference blood cell conditions. Variances were greater in patients. Patient cells exhibited more damage than blood reference group in all experimental conditions. Besides, greater variances in patients indicate that individual variation was present. Sensibility of Comet assay was able to detect DNA damage and to distinguish between patient damage values.

Genetic risk factors for Post-Infectious IBS in the Walkerton outbreak of waterborne gastroenteritis. *AC. Villani[1], M. Lemire[2], M. Thabane[3], SM. Collins[3], D. Franchimont[1], JK. Marshall[3]* 1) McGill University, Canada; 2) Ontario Institute for Cancer Research, Canada; 3) McMaster University, Canada.

In May 2000, heavy rainfall washed livestock fecal residue from nearby farms into inadequately chlorinated water of Walkerton, a small Canadian rural town. Municipal water was contaminated with E. coli 0157:H7 and Campylobacter species, and over 2300 residents developed acute gastroenteritis (GE) with some describing post-infectious irritable bowel syndrome (PI-IBS) symptoms. Although family and twin studies support a genetic basis for IBS, no susceptibility loci have yet been identified. Method: We selected 78 reported functional variants for screening on the Walkerton cohort (*Gastroenterology* 2006;131:445-50), a well characterized longitudinal study. Candidates were classified into 3 categories:1) serotoninergic pathway; 2) intestinal epithelial barrier; and 3) innate immunity risk variants. Variants were genotyped using Sequenom hME or Taqman assays. Analysis consisted at comparing Walkerton residents who experienced GE and reported PI-IBS 2-3 years after the outbreak (220 cases) to those with GE who did not develop PI-IBS (874 controls). A quasi-likelihood association test for cases and controls that accounts for known relatedness within the sample was used. Results: Four candidates show association with PI-IBS. Two are located in TLR9: rs352139 (coding, P545P) (p=0.0135) and rs5743836 (promoter,-T1237C) (p=0.0324) (r^2<0.14); one in CDH1: rs16260 (promoter,-C160A) (p=0.0352); and one in IL-6: rs1800795 (promoter,-G174C) (p=0.0489). Denser mapping was conducted in these 3 regions. We observed two novel associations in IL-6 (p=0.0012) and 14 additional associations that can be explained by LD with the 4 original variants. All SNPs were more significantly associated in the clinically confirmed GE subgroup (p as low as 4.7×10^{-5}), suggesting a clinical gradient. Conclusion: This is the first population study of its kind studying genetic risk factors for PI-IBS. These results indicate abnormalities in genes encoding epithelial barrier functions and innate immune responses to enteric bacteria, and suggest a genetic predisposition to the development of IBS following acute GE.

A community-based participatory approach to developing genetics education materials for Hispanic/Latino students and families and the Pacific Islander community. *L. Stark[1], R. Giles[2], J. Johnson[2], S. Eddings[3]* 1) Genetic Science Learning Center, University of Utah, Salt Lake City, UT; 2) Chronic Disease Genomics Program, Utah Department of Health, Salt Lake City, UT; 3) Bach-Harrison, LLC, Salt Lake City, UT.

The Family Genetics Education Through School and Community Partnerships project seeks to develop culturally-appropriate materials. We first worked with the teacher community to develop genetics curriculum materials for fifth grade (inherited traits) and high school (polygenic, multifactorial chronic diseases and family health history). This ensured that the materials addressed the science and/or health Standards teachers are required to teach. We then worked with a Hispanic/Latino Community Advisory Community to adapt these curricula so that they were culturally-appropriate. The Committee also developed ideas for take-home family materials, and multi-country consensus Spanish translations. The materials have been tested with teachers, students and their families from urban Utah schools with high Hispanic/Latino enrollment. Students completed pre/post knowledge tests, as well as feedback surveys about their reactions to the materials and their familys engagement with the take-home materials; teachers completed more detailed feedback surveys. Fifth grade results: Students showed a significant knowledge gain as a result of using the curriculum. Teachers reported that the materials were very effective, and that the Spanish-language family materials led to students and families as well as parents and teachers having conversations about science - and genetics - that had previously not occurred. High school results: Preliminary data indicate high teacher satisfaction with the curriculum but some challenges for students in collecting family health histories. We are working with a Tongan/Samoan Community Advisory Committee to adapt several of the classroom and family activities for use in community education classes. This project is supported by grant U33MC00157 from the Health Resources and Services Administration, Maternal and Child Health Bureau, Genetic Services Branch and the March of Dimes.

Association between FOXE1 and cleft palate. *M. A. Mansilla[1], L. M. Moreno[1], T. Busch[1], S. A. Bullard[1], J. Machida[1], M. E. Cooper[2], A. Jugessur[3], R. T. Lie[3], A. Wilcox[4], K. Christensen[5], P. Chines[6], A. C. Lidral[1], M. L. Marazita[2], J. C. Murray[1]* 1) Univ Iowa, Iowa City, IA; 2) Univ of Pittsburgh, Pittsburgh, PA; 3) Univ of Bergen, Bergen, Norway; 4) NIEHS, Durham, NC; 5) Univ of Southern Denmark, Odense C, Denmark; 6) NHGRI, NIH, Bethesda, MD.

Non syndromic cleft palate (NSCP) is a complex birth defect caused by genetic and environmental factors. It involves the secondary palate and occurs in 1/2000 live births. Previously we reported a major role for FOXE1 in non syndromic cleft lip with or without cleft palate (NSCL/P). Although NSCP and NSCLP have been described as etiologically separate they share some common genetic and environmental contributors. We investigated the role of FOXE1 in the etiology of NSCP. Our study included 476 families with NSCP from Philippines, USA, Denmark and Norway. Twelve SNPs surrounding and in the FOXE1 gene were tested for association. All populations but those from USA showed significant p values in almost all the SNPs tested. A SNP in the FOXE1 gene, rs1867278, showed the most significant p value of 1E-04, when combining the significant populations. Estimates of the attributable risk for this marker ranged from 29 to 34% depending on the population. The most significant haplotype for this data set was rs1867280-rs3021523 that showed a p value of 9E-04. Foxe1 expression was observed at E10.5 and E11.5 in mouse epithelium involved in the fusion between the medial nasal and maxillary processes. Previous reports have shown that mutations in FOXE1 cause the Bamforth-Lazarus syndrome that includes cleft palate. Altogether these evidence shows that FOXE1 is another transcription factor that plays a role in facial morphogenesis and specifically in both NSCL/P and NSCP.

Ongoing Genomewide Association in Familial Intracranial Aneurysm. *T. Foroud[1], D. L. Koller[1], R. Deka[2], D. Lai[1], D. Woo[2], L. Sauerbeck[2], R. Hornung[2], E. S. Connolly[3], C. Anderson[3], G. Rouleau[3], I. Meissner[2], C. Langefeld[3], J. E. Bailey-Wilson[3], J. Huston[3], R. Brown[3], J. P. Broderick[2]* 1) Indiana Univ Sch Medicine, Indianapolis, IN; 2) Univ Cincinnati, Cincinnati, OH; 3) FIA Study Group.

Subarachnoid hemorrhage due to the rupture of an intracranial aneurysm (IA) occurs in 16,000 to 17,000 persons in the U.S. annually and nearly half of affected persons are dead within the first 30 days. Individuals with 1st degree relatives harboring an intracranial aneurysm (IA) are at an increased risk (GRR=2-4) of IA. Families with multiple members having ruptured or unruptured IA were recruited and all available medical records and imaging data were reviewed to classify possible IA subjects. One definite or probable IA subject from each of 270 Caucasian families were selected for genotyping as cases. Genotyping was performed using the Affymetrix 6.0 in a sample of 270 cases and 281 controls. Quality assessment removed SNPs and samples with call rates below 95%, SNPs with deviation in controls of Hardy-Weinberg equilibrium ($p<.0001$), and minor allele frequency below 0.01; the data set retained 742,338 SNPs. The MDS algorithm implemented in PLINK was performed using HapMap samples to identify and exclude individuals with substantial non-Caucasian admixture. The final analytical sample consisted of 250 FIA cases and 278 controls. The test of allelic association in the FIA case-control cohort for the SNP panel resulted in the detection of several SNPs providing strong evidence of association with FIA, which were supported by additional SNPs in the same gene or chromosomal region. These included the *DHCR24* gene region on chromosome 1p32.3 ($p<2x10^{-6}$), the *HNT* gene region at chromosome 11q25 ($p<3x10^{-6}$), and the *ZEB2* gene region at chromosome 2q22.3 ($p<5x10^{-6}$). We also detected multiple SNPs with evidence of association with FIA in intergenic regions at 4q28.3 and 2q35 (both $p<3x10^{-6}$). Additional case and control samples are currently being genotyped, which will enable us to confirm the evidence of association with FIA in these chromosomal regions and pursue additional genotyping and functional studies of these loci.

Global Changes in DNA methylation in Melanoma. *Y. Koga[1], M. Pelizzola[2], AE. Urban[3], A. Molinaro[2], M. Krauthammer[4], S. Ariyan[5], D. Narayan[5], R. Halaban[6], S. Weissman[1]* 1) Genetics; 2) Epidemiology; 3) Cellular and Developmental Biology; 4) Pathology; 5) Surgery; 6) Dermatology, Yale University, New Haven, CT.

DNA methylation is an important component of epigenetic modifications that influences the transcriptional machinery and is aberrant in many human diseases. Several methods have been developed to map DNA methylation for either limited regions or genome-wide. In particular, antibodies specific for methylated CpG have been successfully applied in genome-wide studies. However, despite the relevance of the obtained results, the interpretation of antibody enrichment is not trivial. Of greatest importance, the coupling of antibody-enriched methylated fragments with microarrays generates DNA methylation estimates that are not linearly related to the true methylation level. Here, we present an experimental and analytical methodology to obtain enhanced estimates which better describe the true values of DNA methylation level throughout the genome. We propose an experimental scenario for evaluating the true relationship in a high-throughput setting and a model-based analysis to predict the absolute and relative DNA methylation levels. We successfully applied this model to evaluate DNA methylation status of normal human melanocytes compared to several melanoma cell strains. Despite the low resolution typical of methods based on immunoprecipitation, we show that model-derived estimates of DNA methylation provide relatively high correlation with measured absolute and relative levels, as validated by bisulfite genomic DNA sequencing. Importantly, the model-derived DNA methylation estimates simplify the interpretation of the results both at single-loci and at chromosome-wide levels.

Common deletions of non-coding regions are strongly associated with Crohns disease and body mass index. *S. McCarroll[1,2], A. Elliott[1,2], A. Huett[2], P. Kuballa[2], S. Chilewski[1], A. Landry[2], M. Zody[1], P. Goyette[3], E. Speliotes[1,4], J. Hirschhorn[1,4], J. Rioux[1,3], D. Altshuler[1,2], R. Xavier[1,2], M. Daly[1,2], NIDDK IBD Genetics Consortium, GIANT Consortium* 1) Broad Institute, Cambridge, MA; 2) Massachusetts General Hospital, Boston, MA; 3) Université de Montréal and Montreal Heart Institute, Montreal, Canada; 4) Children's Hospital, Boston, MA.

Human genome structural polymorphism is common and extensive, but its contribution to phenotypes is not yet defined.

Data from genome-wide association studies (GWAS) may contain more information about CNVs than is appreciated. We typed >1,000 common CNVs in HapMap, found most to be in LD with SNPs, identified tagSNPs, and are using these relationships to assess the extent to which CNVs might explain GWAS signals.

Crohn's disease is associated ($p < 10^{-10}$) with SNPs around the autophagy gene *IRGM*, but *IRGM* lacks explanatory coding-sequence variation. We found a common, 20-kilobase deletion polymorphism, immediately upstream of *IRGM* and in perfect LD with the most strongly Crohns-associated SNP. As a result of this deletion, *IRGM* segregates in the population with two distinct upstream sequences. The deletion (Crohns risk) and reference (Crohn's protective) haplotypes of *IRGM* showed distinct expression patterns across tissues and cell lines. Expression levels of *IRGM* regulated autophagy of intracellular bacteria, a physiological process implicated in Crohns.

Meta-analysis of body-mass index (BMI) across GWAS data by the GIANT Consortium identified novel genomic loci associated with BMI. We found that one strong, replicated association ($p < 10^{-8}$) arises from SNPs that tag a common, 45-kilobase deletion of non-coding sequence; the two strongest BMI-associated SNPs immediately flank this 45-kb deletion on both sides and are in perfect LD with it.

These results identify strong candidate causal polymorphisms at two genomic loci implicated in GWAS and suggest that CNVs influence human phenotypes by regulatory mechanisms.

A Novel Deletion Associated with Anophthalmia and Diaphragm Hernia. *R. T. Chao[1], A. Delaney[2], P. Agarwal[3], L. Nevin[4], M. Akana[3], D. FitzPatrick[5], B. L. Black[3], P. Kwok[3], H. Baier[4], A. M. Slavotinek[1]* 1) Department of Pediatrics, UCSF, San Francisco, CA; 2) University of British Columbia, Canada; 3) Cardiovascular Reseach Institute, UCSF, San Francisco, CA; 4) Department of Physiology, UCSF, San Francisco, CA; 5) MRC Human Genetics Unit, Edinburgh, U.K.

Array comparative genomic hybridization is now standard of care in the investigation of idiopathic mental retardation, and its use is becoming widespread in patients with birth defects. We used Affymetrix 100K SNP arrays to examine 15 patients with congenital diaphragmatic hernia (CDH) or Fryns syndrome for copy number variations (CNVs). Using stringent criteria selecting CNVs with a SNP count of 20 or more and a p-value < 1x10-8, we found 8 regions of CNV suggestive of either a deletion or a duplication that had not been previously described. One patient with a p-value of 0 had tetrasomy 12p, an aberration known to cause CDH. A second patient with a p-value of 0 had unilateral right anophthalmia/microphthalmia, R-sided CDH and mild developmental delay. This male had a novel 2.7 Mb deletion at chromosome 18q22.1 which was verified by FISH, but parental studies showed that the deletion was inherited from his normal mother. *In situ* hybridization studies using murine sections showed that one of the deleted genes, *Txndc10*, was expressed in the developing eye, brain, lung, and kidney. We re-sequenced this gene in 66 anophthalmia/microphthalmia patients, and found c.G260A, predicting p.R39Q, in a patient with microphthalmia and retinal coloboma. The sequence alteration was absent in more than 200 control chromosomes. We are currently testing the hypothesis that *Txndc10* is important in eye development using morpholinos to knock down a zebrafish ortholog of *Txndc10*. Taken together, our results suggest that haploinsufficiency for *Txndc10* may perturb eye development because of the anophthalmia in the patient with the deletion, the conserved expression data, and the presence of a second gene alteration in an unrelated patient with microphthalmia. Our results also emphasize the established utility of array methodology for candidate gene investigation.

Microarray based capturing of human genomic loci for high-throughput re-sequencing and application to cancer genome study. *H. Lee, B. O'Connor, B. Merriman, S. Nelson* Dept Human Gen, Gonda 5554, Univ California, Los Angeles, Los Angeles, CA.

We are approaching a new era in the field of Human Genetics equipped with high-throughput sequencing technique which is opening a new venue to personal genomics and accelerating the discovery of new rare variants in different human disorders. However, even though it is not impossible to sequence the 3-billion-base human genome, it is not very practical yet. This imposes on us a need to develop new methodologies to more effectively utilize the high-throughput sequencers. Thus, we are developing a technology using microarrays to capture specific genomic loci that could be re-sequenced through high-throughput sequencers. Currently, using Agilent custom arrays, we are reaching ~60% enrichment which is comparable to the reported results (Hodges et al. 2007; Albert et al. 2007). However, further refinement of the protocol should improve the enrichment and reduce the cost of sequencing by significant factor. As an application of our developing capture technique, we have designed custom arrays targeting ~3,000 cancerous genes retrieved from the Wellcome Trust Sanger Institute Catalogue of Somatic Mutations in Cancer (COSMIC). By re-sequencing paired samples of normal genomic DNA and tumor genomic DNA within an individual, we hope to identify rare mutations that occurred in the individuals normal genomic DNA in the process of initiating the cancer. This study will not only give us new insights in understanding the cancer development by providing deeper mutational landscape of individual cancer samples, but also show the significance of the capture methodology that could be applied to any human disorders.

Mitochondrial gene set analysis of genome-wide association studies of type 2 diabetes and related clinical traits.

A. V. Segrè[1,2,3], R. Saxena[1,2,3], S. Calvo[1,2,3], V. K. Mootha[1,2,3], L. Groop[4], M. J. Daly[1,2,3], D. Altshuler[1,2,3] 1) Broad Institute of Harvard and MIT, Cambridge, MA; 2) Massachusetts General Hospital, Boston, MA; 3) Harvard Medical School, Boston, MA; 4) Lund University, Malm;ö, Sweden.

Mitochondrial dysfunction is observed in diabetic patients, and mtDNA mutations are linked to a rare form of diabetes, however no common DNA variants (SNPs) linked to mitochondrial genes have yet been robustly associated with T2D in genome-wide association studies (GWAS). While replication is typically done for the top ranked SNPs (p<~1e-4), it is feasible that there are SNPs linked to mitochondrial genes that lie below the top ranked SNPs that are truly associated with T2D. By jointly analyzing a set of SNPs linked to functionally related genes, true associations may be detected that on a single SNP level failed to achieve genome-wide significance. Hence, we tested whether mitochondrial genes or the oxidative phosphorylation (OXPHOS) subset are statistically enriched for genes modestly associated with T2D or related clinical traits. We developed a gene set enrichment method for analyzing GWAS, based on similar approaches for expression data. The method involves: (i) assigning to each gene in the genome the highest score among the GWAS scores of all SNPs in a region around the gene, (ii) normalizing the gene scores according to confounders (e.g. gene size), (iii) defining the sets of functionally related genes to be tested, (iv) testing statistically whether any of the gene sets is enriched for high scores, and (v) estimating statistical significance by permutation analysis. We applied our method to a meta-analysis of three GWAS of T2D, using 933 mitochondrial genes and 86 OXPHOS genes. No significant enrichment of gene associations to T2D was found for the mitochondrial or OXPHOS gene sets using an enrichment cutoff of the top 1% or 5% of all ranked genes (p>0.2). Simulations used to assess the power of our method suggest that at least 50 mitochondrial genes need to lie in the top 5% of ranked genes (38 observed) or 8 OXPHOS genes (1 observed) for collective association to be identified at a 0.05 false positive rate.

Scanning for gene-gene interactions involving known type 2 diabetes genes and the genome in 2335 Finnish cases and controls. *T. Hu[1], L. J. Scott[1], L. Bonnycastle[2], N. Narisu[2], M. A. Morken[2], P. A. Deodhar[2], T. T. Valle[3], J. Tuomilehto[3], R. N. Bergman[4], K. L. Mohlke[5], F. S. Collins[2], M. Boehnke[1]* 1) Department of Biostatistics, University of Michigan, Ann Arbor, MI; 2) National Human Genome Research Institute, Bethesda, MD; 3) National Public Health Institute, Helsinki, Finland; 4) University of Southern California, Los Angeles, CA; 5) University of North Carolina, Chapel Hill, NC.

~20 loci have been identified as convincingly associated with the risk of type 2 diabetes (T2D). However, little is known about the interactions among these loci or with other variants across the genome. Since gene-gene interaction may play an important role in determining disease risk, we set out to test the interactions of T2D associated variants in and around identified regions in genome-wide association (GWA) data from the Finland-United States Investigation of NIDDM Genetics (FUSION) study. Based on a sample of 1161 T2D cases and 1174 normal glucose tolerant controls genotyped for >300,000 SNPs, we tested for two-way interactions among the following loci: *IGF2BP2* (rs4402960), *CDKAL1* (rs7754840), *CDKN2A/B* (rs10811661), rs9300039, *FTO* (rs8050136), *PPARG* (rs1801282), *SLC30A8* (rs13266634), *HHEX* (rs1111875),*TCF7L2* (rs7903146), *KCNJ11* (rs5219), *JAZF1* (rs864745), *CDC123* (rs12779790), *TSPAN8* (rs7961581), *THADA* (rs7578597), *ADAMTS9* (rs4607103), and *NOTCH2* (rs10923931), using logistic regression and controlling for age, sex, and birthplace. None of these two-way interactions gave results sufficiently significant to survive correction for the 120 correlated tests. We currently are using this same strategy to test for interaction between the T2D-associated loci and the >300,000 SNPs genotyped in our FUSION GWA.

Cross-species targeted DNA sequencing: New methods for exploring diversity and evolution. *M. Lovett* Dept Genetics, Washington Univ Sch Med, St Louis, MO.

Cross-species DNA sequencing presents particular technological challenges. PCR primers designed from a reference sequence frequently do not amplify DNA from other species. We have developed methods to achieve large-scale, cross-species sequence comparisons and have applied these to regions within the genomes of six Darwins finch species plus out groups. Up regulation of *BMP4* gene expression is the only difference discovered to date correlated with large beak morphology in these birds. It is possible that differences in a trans-regulator of *BMP4*, or different cis-regulatory sequences between the species, account for these differences. To test the latter we targeted the 500kb region surrounding the *BMP4* gene for DNA sequencing in the finch species. This was achieved by direct genomic selection (DIGS) using a biotinylated *Gallus gallus* BAC contig. The published DNA sequence over this region has many gaps, including the putative *BMP4* promoter. We therefore also sequenced the entire region in leghorn chicken DNA to fill these gaps. Genomic DNAs were modified by shearing and the addition of Illumina/Solexa sequencing linkers. Hybridization selections were conducted in solution and captured and eluted DNAs were directly sequenced. Approximately 20% of chicken reads mapped to the reference and these provided close to 100% coverage of single copy sequences in the contig at 100X coverage. This filled in the missing gaps in the *Gallus gallus* sequence including the putative *BMP4* promoter. Assembly of the finch DNA sequences readily identified 20% of the contig as having high levels of sequence conservation between chickens and finches, with the remainder of the contig showing much higher conservation within the finches than with the chicken. Within the assembled contigs are numerous examples of conserved elements, including the promoter of the *BMP4* gene and many other more distal elements, some of which differ between finch species. These are being functionally assessed. This is the first example of any large- scale DNA sequence comparison between Darwins finches and has applications for DNA sequencing of large regions within many other species.

DNA damage produced following UV radiation in combination with hydrogen peroxide in skin cells. *J. Perron, N. Bastien, JF. Millau, R. Drouin* Genetics, Dept Pediatrics,, Fac Med Health Sci, Univ Sherbrooke, Sherbrooke, Quebec, Canada.

The main etiological factors responsible for skin cancer are the ultraviolet radiations emitted by the sun. Indeed, long wavelength UVB (295-320 nm) and UVA (320-400 nm) reach the skin surface and cause DNA damage like cyclobutane pyrimidine dimers (CPD) and oxidized bases. These damage lead to mutations and ultimately to cancer. In addition, other cellular molecules can also interfere within this process such as hydrogen peroxide (H2O2) produced during skin inflammation. The objective of this study was to compare the formation of damage in human fibroblasts following UV irradiation, hydrogen peroxide treatment or a combination of both. Methods- We irradiated fibroblasts with UVA, UVB or solar simulated light (SSL) with or without different concentrations of H2O2. DNA damage were converted in single-strand breaks using the T4 endonuclease V to study CPD and the Endonuclease III or Formamidopyrimidine DNA glycosylase to study oxidative damage. Then, glyoxal gels were performed to assess the global frequency of DNA damage. Finally using LMPCR technique (ligation-mediated PCR) on the p53 gene we mapped DNA damage formation at the level of nucleotide resolution. Results- In fibroblasts, the combination of UVB and H2O2 did not induce a higher frequency of damage than UVB and H2O2 alone but a combination of UVA and H2O2 led to increased levels of oxidative damage comparatively to UVA or H2O2 alone. The CPD were the most important photoproducts following UVA irradiation. The CPD were mapped at the dipyrimidine sites, mainly the TT sites. The oxidative damage were mapped mainly at the G residues suggesting the formation of 8-oxo-guanine. Conclusion- UVB are known to be implicated in skin cancer but the contribution of UVA in the sun induced carcinogenesis process is still unclear. These results show that UVA could play an important role in the formation of mutations, particularly if their effect is combined with the H2O2 produced during the sunburn inflammatory response. Moreover we also showed that UVA produced CPD, reinforcing the potential role of UVA in skin cancer genesis.

Loss-of-function mutations in endothelial lipase are associated with elevated HDL cholesterol in humans. *A. Edmondson[1], R. Brown[1], S. Kathiresan[2], L. A. Cupples[3], S. Demissie[3], A. Manning[3], E. Rimm[4], J. Wang[5], M. Wolfe[1], S. DerOhannessian[1], D. Evans[6], R. Hegele[6], D. J. Rader[1]* 1) Institute for Translational Medicine and Therapeutics, University of Pennsylvania School of Medicine, Philadelphia, PA, USA; 2) Broad Institute of Harvard and MIT, Cambridge, MA, USA; 3) Boston University and Framingham Heart Study; 4) Department of Epidemiology, Harvard School of Public Health, Boston, MA, USA; 5) Robarts Research Institute and Schulich School of Medicine and Dentistry, University of Western Ontario, London, Ontario, Canada; 6) Endokrinologie und Stoffwechsel, Medizinische Klinik III, Zentrum für Innere Medizin, Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany.

Elevated plasma levels of high density lipoprotein cholesterol (HDL-C) are associated with protection from cardiovascular disease. Animal models indicate that increased expression of endothelial lipase (*LIPG*) is inversely associated with HDL-C levels, and genome-wide association studies have identified *LIPG* as being associated with human HDL-C. We hypothesized that loss-of-function mutations in *LIPG* are a cause of elevated HDL-C and performed medical resequencing of *LIPG* in cases with HDL-C > 95th percentile and controls with HDL-C < 25th percentile. We identified an excess of nonsynonymous *LIPG* variants in cases with elevated HDL-C. Functional analysis using in vitro lipase activity assays demonstrated that the unique nonsynonymous *LIPG* variants identified in cases with elevated HDL-C exhibited significantly decreased lipase activity. The association of two more common nonsynonymous *LIPG* variants, T111I and N396S, with HDL-C levels in the Framingham Heart Study was studied and indicated that N396S was significantly associated with increased HDL-C (p=0.00004), while T111I was not associated with HDL-C. Functional analysis demonstrated that N396S has significantly decreased in vitro lipase activity, while T111I has normal in vitro lipase activity. Our results establish that loss-of-function mutations in *LIPG* lead to increased HDL-C levels and support the concept that endothelial lipase is an attractive candidate for inhibition to raise HDL-C.

The DISC1 locus - A circumscribed intron 9-interval associates with disease risk in females affected by schizophrenia. *J. Schumacher[1, 2], G. Laje[2], R. Abou Jamra[1], T. Becker[3], T. W. Muehleisen[4], C. Vasilescu[4], M. Mattheisen[4], S. Herms[4], P. Hoffmann[4], A. M. Hillmer[4], A. Georgi[5], C. Herold[3], T. G. Schulze[2, 5], F. J. McMahon[2], P. Propping[1], M. Rietschel[5], M. M. Noethen[1, 4], S. Cichon[1, 4]* 1) Institute of Human Genetics, University of Bonn, Germany; 2) National Institute of Mental Health (NIMH), National Institutes of Health (NIH), Bethesda, USA; 3) Institute for Medical Biometry, Informatics and Epidemiology, University of Bonn, Germany; 4) Department of Genomics, Life & Brain Center, University of Bonn, Germany; 5) Division of Genetic Epidemiology in Psychiatry, Central Institute of Mental Health, Mannheim, Germany.

The gene Disrupted-In-Schizophrenia-1 (DISC1) has been reported as a risk factor for schizophrenia by association studies. It encodes a multifunctional scaffold protein involved in neuronal migration, cortical layering, and hippocampal formation; all processes implicated by the neurodevelopmental hypothesis of schizophrenia. We conducted a systematic LD mapping at the DISC1 locus using 556 polymorphisms (121 genotyped, 435 imputed SNP markers) in a case-control-sample from the German population (N=1,621, cases=782, controls=839). To protect from type-I error we performed 10,000 permutations. A circumscribed DISC1 intron 9-interval was significantly overrepresented in schizophrenia female patients (38% vs. 28% in controls, p=4x10-5), spans between 27.5-28.8 kb at the genomic level, and is associated to early onset (< 21 years) (44% vs. 28%, p=9x10-5). The corresponding ORs are in the range of 1.46 to 1.88. Our results point to a so far undescribed DISC1 susceptibility interval that may contribute to the disease risk in the German (or mid-European) population. However, other DISC1 intron 9 associations with schizophrenia have already been reported. This, together with the high cross-species conservation and the exceptionally large size of intron 9 suggest that this interval might be of functional importance in the disease process. Furthermore, based on present and previous findings, the consideration of allelic heterogeneity and gender-specific effects seems to be crucial in genetic studies of schizophrenia.

Isolating genetic causes of familial lung cancer. *C. I. Amos[1], J. E. Bailey-Wilson[2], S. M. Pinney[3], A. G. Schwartz[4], M. You[5], P. Yang[6], G. Gaba[7], D. Mandal[8], P. Fain[9], Y. Li[1], J. Minna[10], E. Kupert[3], M. deAndrade[6], M. W. Anderson[3]* 1) Dept Epidemiology, MD Anderson Cancer Ctr, Houston, TX; 2) National Human Genome Research Institute; 3) University of Cincinnati, Cincinnati; 4) Karmanos Cancer Institute, Detroit; 5) Washington University, St. Louis; 6) Mayo Clinic College of Medicine, Rochester, MN; 7) Medical College of Ohio, Toledo; 8) Louisiana State University Health Sciences Center, New Orleans; 9) University of Colorado, Denver; 10) U.T. Southwestern Medical Center, Dallas.

Individuals with a first degree relative with lung cancer are at approximately a 2.5 fold higher risk for lung cancer compared with population rates that allow for smoking behaviors. We previously identified in 2004 by linkage analysis a region of chromosome 6q that harbors a susceptibility locus for lung cancer using data from 52 families with at least three relatives who had lung cancer. Previously we found that families with 5 or more affected relatives showed the strongest evidence for linkage, yielding a heterogeneity LOD (HLOD) score of 4.26. Our current studies expand to 92 the number of families that we have studied to identify a strongly familial cause for lung cancer, and increase the time of observation and number of cases with lung cancer. Evidence for a lung cancer susceptibility locus on chromosome 6q is strongly supported in families that include 5 or more affected relatives in 2 or more generations, yielding an HLOD score of 4.57 at 158 cM. Genetic linkage analysis of other chromosomes provided weaker evidence for linkage. Evidence for linkage is also provided in these families to chromosomes 6p (HLOD score of 1.72 at 63 cM), 4p (HLOD score of 1.3at 4cM) and chromosome 12 (HLOD score of 0.97 at 146 cM). Further analyses of the risk for lung cancer from the chromosome 6q locus show a dramatic increase in risk among carriers who smoke any amount, compared with nonsmokers. These results strongly support evidence for at least one locus on chromosome 6q that greatly increases risk for lung cancer particulary in response to smoking, and suggest that additional loci contribute to lung cancer risk.

Deep sequencing of genes associated with type 2 diabetes and Crohn's disease. *M. Daly[1,2,3], F. Kuruvilla[1,2], N. Burtt[2], C. Stevens[2], C. Guiducci[2], G. Crawford[2], J. Maguire[2], K. Cibulskis[2], T. Green[1,2], M. Rivas[1,2], B. Voight[1,2], E. Lander[1,2,3], S. Gabriel[2], D. Altshuler[1,2,3], NIDDK IBD Genetics Consortium; Diabetes Genetics Initiative* 1) CHGR, Massachusetts General Hospital, Boston, MA; 2) Broad Institute of Harvard and MIT, Cambridge, MA; 3) Harvard Medical School, Boston, MA.

Genome-wide association studies have identified more than 100 unequivocally significant associations to complex diseases. In most cases, specific causal variants have yet to be pinpointed, limiting follow-up functional studies and even recognition of which nearby gene is involved. Moreover, since the proportion of disease risk explained is generally quite modest, deeper assessment of variation is further motivated by observations of multiple independent risk alleles at many susceptibility loci (e.g., IRF5, IL23R, NOD2) and particularly the observation of both rare variants of large effect and common variants of modest effect at many genes conclusively linked to lipid levels. Thus we have undertaken deep sequencing of coding regions of 50 genes near SNPs identified and replicated beyond genome-wide significance in Type II Diabetes and Crohns Disease using Solexa/Illumina technology. Since the PCR-targeted exons are small relative to the capacity of a lane of Solexa, we sequenced 500 cases and controls in pools of 50 individuals. To identify SNPs we implemented a likelihood-based estimate of p(data | genotype) with population genetics priors on polymorphism rates informed by HapMap and dbSNP variant locations. By targeting a set of previously typed rare variants, we observed 90% sensitivity to detect rare variants carried by 1 of 100 chromosomes, suggesting pooling and rare SNP detection were successful. Validation genotyping identified a modest but inflated number of false positives - automated analysis of neighborhood quality further refined SNP discovery. We present here the experimental and analytic strategy and apply it to summarize rare coding variation and aggregate excesses in cases versus controls - providing an overall assessment of the role of rare variation in genes identified through common SNP association.

High Resolution Comparative Genomic Hybridization of pre-invasive laser-capture microdissected lung adenocarcinoma. *L. Herlitz, A. C. Borczuk, O. Nahum, C. A. Powell, B. Levy* Department of Pathology, Columbia University, New York, NY.

Non-mucinous bronchioloalveolar carcinoma (BAC) is pre-invasive lung adenocarcinoma characterized by tumor growth along benign alveolar walls. DNA derived from homogenized tumor sections contains a large proportion of non-tumoral cells. Laser capture microdissection (LCM) allows for significant enrichment of tumor cells improving detection of small copy number changes and deletions. LCM of tumor cells from frozen sections of 7 cases of non-mucinous BAC was performed using the PALM-Zeiss Microbeam System. Whole genome amplified DNA was used for metaphase spread comparative genomic hybridization. Increases in DNA copy number were observed in chromosomes 1q32, 5p, 7p, 8q24 and losses in 9p13-21, 11q13 in at least 5 of the 7 cases. Known cancer genes in these regions include KIF4, CENPF, KCNH1, EGFR,RECQL4, RNF139 and tumor suppressor genes p16 and MEN1. LCM allows for the detection of copy number alterations in tumors in which less than 50% of the cut surface represents tumor cells. This creates the opportunity for uncovering genes that play a role in the etiology of early neoplasia and pre-invasive lesions.

Lysosomal Disease Network, and *WORLD Symposium 2009*. *C. Whitley[1], J. Barranger[2], J. Muenzer[3], C. Eng[4], G. Grabowski[5], M. Patterson[6], S. Walkley[7], B. Davidson[8], R. Steiner[9], W. Wilcox[10], E. Shapiro[1], . other LDN members[11]*
1) University of Minnesota, Minneapolis, MN; 2) University of Pittsburgh, Pittsburgh, PA; 3) University of North Carolina; 4) Baylor College of Medicine, Houston, TX; 5) Children's Hospital Research Foundation, Cincinnati, OH; 6) Mayo Clinic, Rochester, MN; 7) New York University, New York, NY; 8) University of Iowa, Iowa City, IA; 9) Oregon Health & Science University, Portland, OR; 10) Cedars-Sinai Medical Center, Los Angeles, CA; 11) other LDN institutions.

The Lysosomal Disease Network (LDN) is a consortium of basic researchers, clinicians, pharmaceutical industry professionals, and patient advocates devoted to promote and facilitate basic, translational and clinical research in lysosomal diseases. The network is a scalable multi-center consortium of geographically distributed expert medical centers, patient support and corporate partners. The infrastructure, mission and charter were developed through organizational committees; the collaborating medical centers, pharmaceutical and patient support organizations will direct the activities of the network through participation in the Steering Committee. Toward meeting its goals, the LDN has launched its web site **www.LysosomalDiseaseNetwork.org** to facilitate professional and public education, timely network communication, and develop a data entry mechanism for research projects. The web site has announced its 5th annual scientific meeting, the **WORLD Symposium** (February 18-20, 2009) in San Diego, CA, USA. Through the web site, participants are able to submit abstracts, register for the meeting, and join the growing membership list to receive regular communications. The Network is currently developing longitudinal studies to understand the natural history of lysosomal diseases and treatment outcomes. Long term network goals include: 1) development of an information management infrastructure to encourage integration of shared clinical experience and relevant longitudinal studies, 2) investigator training, and 3) public education. Organizational and symposium support has been provided by NIH, NINDS, NIDDK, and ORD.

THE HUMAN VARIOME PROJECT - Collection of variation causing human disease: Progress Pilots and Plans. *J. T. Den Dunnen[1], F. A. Macrae[2], R. G. H. Cotton[3], Collaborators of the HVP, HGVS and InSiGHT* 1) Human Genetics, S4-030, Leiden Univ Medical Ctr, Leiden, Netherlands; LOVD; 2) Department of Colorectal Medicine and Genetics, The Royal Melbourne Hospital, Parkville, Australia; InSiGHT; 3) Genomic Disorders Research Centre, Melbourne, Australia; Human Variome Project; Department of Medicine, The University of Melbourne.

Lack of up-to-date, complete, electronically accessible and correctly curated information on gene sequence variants leads to excessive web searching, wastes valuable healthcare funds and may lead to incorrect clinical diagnosis. The Human Variome Project (HVP; www.humanvariomeproject.org) was created to coordinate the curation and collection of all genetic variation, its phenotype and associated disease(s). A planning meeting was held in Spain (May 2008) to focus the task. A number of projects are planned or underway under the HVP or related and/or collaborator activities. These include: 1. The HVP InSiGHT colon cancer pilot study to set up a trial system in inherited colon cancer genes (HNPCC) to be later applied to all genes in all countries. 2. The microattribution initiative initiated by Myles Axton, Nature Genetics. 3. Ethics of curation of gene or locus specific databases (LSDBs). 4. GEN2PHEN, a European initiative to rationalize and harmonize databases. 5. Development of systems to collect data from hospitals and diagnostic labs. Country-specific efforts are underway in the UK and Germany and planned in Japan and the Arab countries. 6. Establishing data exchange between LSDBs and central repositories like NCBI and EBI. 7. Locus specific database hosting, registering and software are all available.

Clinical Ascertainment of Patients with Muir-Torre Syndrome by Dermatologists and Pathologists within a Large HMO. *K. Wendt[1], S. A. Ahmed[1], F. Eggerding[2], A. Thomas[1], M. Jamehdor[1]* 1) Regional Gen Testing Lab, Kaiser Permanente SCPMG, Los Angeles, CA; 2) HMRI, Pasadena, CA.

Muir-Torre syndrome (MTS) is characterized by sebaceous skin lesions (adenomas, epitheliomas, basaliomas and carcinomas) and one or more visceral malignancies (colorectal, endometrial, ovarian, stomach) within the spectrum of hereditary nonpolyposis colorectal cancer (HNPCC). A subtype of MTS allelic to HNPCC is caused by germline mutations in the DNA mismatch repair (MMR) genes, leading to early onset colorectal cancer and other tumors. Both microsatellite instability (MSI) testing and immunohistochemical examination of MMR protein expression in MTS-associated skin tumors can be used as a diagnostic screening tool to identify patients with MMR germline mutations. During a 3 yr period (2/2005-6/2008), the SCPMG Genetic Testing Laboratory received 23 samples (6 blood samples, 17 paraffin-embedded sebaceous adenoma tumors) from 22 patients (age 45-87y; 13M: 9F) at risk for MTS. Each of the 6 blood samples for MMR germline testing was submitted by one of our Medical Geneticists based upon their patients personal history of sebaceous adenoma and family history of colon or other HNPCC-related malignancy. Of the 17 paraffin-embedded sebaceous adenoma tumor samples received, 14 were submitted by Pathologists and Dermatologists within our HMO. In 6 of 14 cases, absent immunostaining for MSH2/MSH6 was noted. Microsatellite instability (MSI-H) was noted in 5 of the 6 cases with absent MSH2/MSH6 immunostaining. Although most HNPCC cases are ascertained through Medical Geneticists and/or Gastroenterologists, our report acknowledges the role of Dermatologists and Pathologists in raising the suspicion of HNPCC and initiating MSI/IHC testing following diagnosis of a sebaceous neoplasm. The unique structure within our HMO allows us to review abnormal MSI/IHC results with referring Dermatologists and Pathologists and re-integrate those patients into our genetic service. Patients are referred to their local Medical Geneticist and Genetic Counselor for evaluation of the extended pedigree, consideration of germline testing and coordination of surveillance for MTS-associated visceral and skin neoplasms.

Genome-wide association study reveals *ADIPOQ* variation as strongest genetic influence on circulating adiponectin levels in the Old Order Amish. *T. I. Pollin[1], H. Ling[1,2], P. F. McArdle[1], Q. Gibson[1], B. D. Mitchell[1], J. R. O'Connell[1], A. R. Shuldiner[1]* 1) University of Maryland School of Medicine, Baltimore, MD; 2) Center for Inherited Disease Research, Baltimore, MD.

Adiponectin is a fat-secreted hormone that enhances insulin sensitivity and free fatty acid oxidation and inhibits inflammation. Reduced levels are associated with obesity and increased risk of type 2 diabetes and cardiovascular disease. We measured adiponectin levels in 689 Old Order Amish participants of the Heredity and Phenotype Intervention (HAPI) Heart Study and performed a genome-wide association study using the Affymetrix GeneChip Human Mapping 500K Array. The greatest evidence for association with ln-transformed adiponectin levels occurred at three SNPs on chromosome 3q27 in or near *ADIPOQ*, the adiponectin structural gene, including rs3774261 in intron 2 and rs6773957 in the 3' UTR (minor allele frequency [MAF] = 0.31, additive $p = 8.1 \times 10^{-8}$ for both, adjusted for sex and sex-specific age and age^2 as well as residual covariance between relatives), and rs698092, 393 kb downstream (MAF = 0.33, $p = 1.6 \times 10^{-8}$). The two *ADIPOQ* SNPs (both C>T) were in complete LD (D' = 1, $r^2 = 1$), and adiponectin levels (geometric mean [95% CI]) were 9.7 [9.4 - 10.1], 10.4 [10.1 - 10.8] and 12.1 [11.4 - 13.0] mg/dl for the CC, CT and TT genotypes respectively. Either SNP accounted for 5.0% of trait variation. Rs698092 was in low LD with the two *ADIPOQ* SNPs (D' = 0.16, $r^2 = 0.03$), explained 4.2% of the trait variance and may tag a distinct causal variant in *ADIPOQ*. A prior genome-wide linkage study in the Amish Family Diabetes Study (AFDS) also identified the *ADIPOQ* locus as the greatest genetic predictor of adiponectin levels (LOD = 2.13). Five additional regions contained SNPs associated at $p 10^{-5}$: rs1290894 at 3q13 (MAF = 0.05), rs1380722 at 4q32 (MAF = 0.05), rs996825 at 8q12 (MAF = 0.16), rs1857471 at 9q21 (MAF = 0.05), and rs2420941 at 10q26 (MAF = 0.46). These findings confirm the key influence of *ADIPOQ* variation on adiponectin levels and provide opportunities for elucidating additional genetic influences on adiponectin levels and associated diseases.

Association of the Monoamine oxidase gene A and autism spectrum disorder. *L. Qi[1,6], F. Tassone[2], W. Zhang[1], P. Krakowiak[4,6], R. Hansen[3,6], I. Hertz-Picciotto[4,6], I. Pessah[5,6]* 1) Program Human Gen, Univ California, Davis, Davis, CA; 2) Department of Biochemistry and Molecular Medicine, School of Medicine, Univ California, Davis, CA; 3) Dept of Pediatrics, School of Medicine, Univ California, Davis, CA; 4) Dept of Public Health, School of Medicine, Univ California, Davis, CA; 5) Dept of Molecular Biosciences, School of Medicine, Univ California, Davis, CA; 6) M.I.N.D. Institute, UCD Medical Center, Sacramento, California.

We have studied three polymorphisms, the serotonin transporter promoter region polymorphism (5-HTTLPR), the dopamine hydroxylase (DBH) and the Monoamine Oxidase A (MAOA) in families participating in the CHARGE (Childhood Autism Risks from Genetics and the Environment) Study. The CHARGE study is a large ongoing case-control investigation which recruits families with a child aged 2-5 years from three groups: autism, ASD, and the general population. A total of 359 AU, ASD and TD families were genotyped to investigate whether allelic variations in the candidate genes are associated with higher risk of autism and whether transmission differed by autism case status. Case-control association analysis shows that allele 4 in MAOA doubled the risk for AU or AU +ASD among White and Hispanic boys (95% CI = 1.10, 3.59, p = 0.02 for AU and 95% CI = 1.18, 3.49, p = 0.01 for AU+ASD), with or without adjusting for other covariates. Family-based association analysis does not show significant transmission disequilibrium for HTT, DBH, and MAOA. However, more mothers had the 44 genotype among AU (49%;) and AU&+ASD (45%) boys than among TD boys (31%, p = 0.005 for AU vs TD and p = 0.01 for AU+ASD vs TD, using White and Hispanic subjects). Moreover, mothers MAOA genotype is significantly associated with risk for AU or AU+ASD ($p < 0.05$). Adjusted for race, mothers and fathers age at delivery, mother having the 34 or 44 genotype is associated with 2 to 4.7-fold increase in the risk of AU or AU+ASD compared with the genotype 33. These results provide more evidence that allele 4 in MAOA may be a risk allele for Autism Spectrum Disorders.

Plasma Peptide Tyrosine Tyrosine Levels are Increased in Urea Cycle Disorder Patients. *S. Mitchell, D. Murdock, M. Summar* Center for Human Genetics Research, Vanderbilt University, Nashville, TN.

Peptide tyrosine tyrosine (PYY) and N-acetylglutamate synthase (NAGS) are divergently transcribed and separated by less than 200 base pairs in humans; an arrangement that is consistent with coordinate regulation. PYY inhibits gut motility and is a key component in the regulation of appetite, inducing feelings of satiety postprandially. NAGS plays an important role in ureagenesis providing the necessary co-factor for carbamoyl phosphate synthetase 1, which catalyzes the first step of the urea cycle. In the urea cycle, ammonia is converted to urea and is excreted as waste through the kidneys. Inherited metabolic disorders resulting in deficiencies in any of the urea cycle enzymes result in aberrant nitrogen clearance and elevated ammonia levels. Additionally, anorexia is well-documented in patients with urea cycle disorders (UCDs). Decreased appetite is normally attributed to cerebral edema and dysregulation of neurotransmitters resulting from the elevations in ammonia levels. However, if PYY and NAGS are regulated in a coordinate manner due to their arrangement and physical proximity, then increased levels of PYY may underlie a significant part of the anorexia observed in hyperammonemic patients. We hypothesize that patients with UCDs and an upregulation of NAGS will show increases in PYY production. Using an ELISA, we measured plasma PYY levels in a group of UCD patients (n = 37). The levels ranged from 10.5 pmol/L to 249.5 pmol/L, and the average level was 48.8 pmol/L. The upper end of this range is dramatically higher than ranges reported for normal fasting and postprandial plasma PYY levels, 12.1 pmol/L and 29.9 pmol/L, respectively. We plan to further investigate these preliminary results; as the increased expression of PYY may result in appetite suppression in these patients leading to catabolism, and an increase in ammonia levels. If clinical observations in UCD patients are directly relevant to the transcriptional regulation of this gene pair, then PYY becomes a potential therapeutic target in the management of patients suffering from UCDs and other diseases involving disrupted nitrogen metabolism.

Validation of the manual scanning process to detect rare events using FISH technique. *A. Emad, S. Ayub, MC. Gregoire, O. Samassekou, M. Gadji, F. Hemmings, K. Krabchi, R. Drouin* Genetics, Dept Pediatrics,, Fac Med Health Sci, Univ Sherbrooke, Sherbrooke, Quebec, Canada.

Introduction- Fetal cells are found in the maternal circulation at a rare frequency of 2-6 cells/ml. The male fetal cells by serving as markers XY are a potential source of developing non-invasive prenatal diagnosis. Techniques like Fluorescence In Situ Hybridization (FISH) and Primed In Situ labeling (PRINS) have been used before for retrieval of these cells. To our knowledge, the efficiency of the manual scanning using either of these techniques has never been evaluated. Objectives- For the purpose of developing non-invasive prenatal diagnosis, we are in the process of determining the efficiency of the manual scanning by the FISH technique to detect rare XY cells amongst thousands of XX cells. Hybridization efficiency is also determined. Methodology- The methodology involves spreading of 1-10 XY cells on pre-cleaned slides, staining with Giemsa, imaging of these cells then spreading around 100,000 XX cells, followed by FISH using XY probes. This was followed by blind manual scanning with the Y chromosome serving as marker. Pictures taken of XY cells are compared with the ones taken initially following Giemsa staining. Results- A total number of 76 XY cells were distributed on 17 slides processed by manual scanning using FISH technique. The scanner was able to detect 66 of these XY cells. By evaluation of the hybridization of the 10 missed cells, it was found that 20%; (2/10) were not hybridized for the Y chromosome, 30%; (3/10) were poorly hybridized and the hybridization was adequate at the remaining 50%; (5/10). The overall detection rate after exclusion of the cells with defective hybridization was 92.9%; (66/71). Conclusion- Manual scanning of the slides processed by FISH can lead to underestimation of the real number of cells by about 13.1%; (10/76). FISH technique is responsible for missing of 2.6%; (2/76) while 6.6%; (5/76) is missed in the process of the manual scanning itself. The rest 3.9%; (3/76) is in a gray zone in-between. This data is mandatory for later comparison between different techniques on one hand and manual and automatic scanning on the other hand.

Haplotype analysis enabled the dissection of two chromosome 2 variants with major effect on plasma plant sterol levels on the Pacific Island of Kosrae. *E. Kenny[1,2], A. Gusev[2], D. Lütjohann[3], J. Lowe[1,4,5], J. Salit[1], J. Maller[4,5,6], M. Stoffel[1], M. Daly[4,5,7], D. Altshuler[4,5,7], J. Friedman[1,8], I. Pe'er[2], J. Breslow[1], E. Sehayek[9]* 1) Div Biol, 156-29, Rockefeller Univ, New York, NY; 2) Columbia University, New York, NY; 3) University of Bonn, Germany; 4) Broad Institute of Harvard & MIT, Cambridge, MA; 5) Massachusetts General Hospital, Boston, MA; 6) University of Oxford, Oxford, UK; 7) Harvard Medical School, Boston, MA; 8) Howard Hughes Medical Institute; 9) Cleveland Clinic, Cleveland, OH.

Plasma plant sterol levels (PPS) serve as a surrogate measure of cholesterol absorption from the intestine. Measurement of PPS on the Pacific Island of Kosrae previously identified five -sitosterolemic patients homozygous for a frame shift mutation in ABCG8 on chr2p21 and 11% of the islanders who are heterozygous for the ABCG8 mutation and have 30-50% increased PPS. To characterize this and other genetic determinants of PPS, we performed a genome-wide association study in 1,429 related individuals. As expected, a very strong association of PPS with SNPs was detected on chr2p21 ($p<10^{-40}$). Association analysis that has been conditioned on the ABCG8 mutation disclosed a second strong signal that maps ~250kb downstream of the ABCG8 locus ($p<10^{-35}$). Identity-by-descent analysis of the population (using the GERMLINE software) revealed that the entire downstream signal was captured by a 525kb haplotype (chr2:43.8-44.3MB) that harbors the ABCG8 locus but was completely unlinked to the ABCG8 mutation. The novel haplotype is carried by ~2% of the population, and segregates in two large kindreds of the same Kosraean village. Carriers show >90% increase in PPS ($p<10^{-69}$) which exceeds the ABCG8 mutation effect. Carriers of the novel haplotype are characterized by 50% decrease in cholesterol synthesis, as reflected by a decrease in plasma lathosterol levels ($p<10^{-11}$). We are currently resequencing search for the causative genetic variant on this haplotype. These findings exemplify the power of haplotype analysis in dissecting the effect of multiple variants of the same locus.

HEMIZYG: Detects Novel SNP in Bipolar Linkage Region And Uncovers Evidence for Genetic Heterogeneity.

W. C. L. Stewart[1], N. Xiang[3], S. Villafuerte[3], S. Zoellner[1,2], M. G. McInnis[2], M. Burmeister[3] 1) Dept Biostatistics, Univ Michigan, Ann Arbor, MI; 2) Dept Psychiatry, Univ Michigan, Ann Arbor, MI; 3) Dept Human Genetics, Univ Michigan, Ann Arbor, MI.

The impact of novel genetic variants (e.g. rare alleles and/or deletions) on common, complex disesaes is largely unknown as these variants are often missed by genome-wide discovery efforts. To increase the rate at which novel variants are discovered, we exploit the fact that these variants tend to have large effects on marker-specific traits (e.g. genotype signal intensities) to confirm the existence of a novel SNP in a 20 Mb region of 8q24. This region was recently identified in a meta-analysis of 11 bipolar (BP) studies as a candidate region for BP disorder. In our data, which consist of 737 multiplex BP families genotyped at 1536 SNPs using Illumina Bead Array technology, we hypothesized the existence of a novel variant that negatively affects the genotyping assay at SNP rs2978607. We used the deletion detection program HEMIZYG to impute the genotypes at the novel SNP from the genotype signal intensities at the known SNP (rs2978607), which led to the successful identification of 100 true carriers of the novel allele (confirmation obtained via pyrosequencing). We find insufficient evidence to reject the null hypothesis of no association between the novel SNP and BP disorder; however, there is evidence to support the presence of a second novel variant, and this variant may increase risk for BP disorder ($p \sim 0.001$). We examined this possibility by selecting only those families that did not segregate for the apparent second novel variant. Consistent with a pattern of genetic heterogeneity, the maximum nonparametric LOD (NPL) score increased from 0.63 in the original 737 families to 1.17 in the selected set. By contrast, random subsets of the same size yield, on average, a decrease in the NPL score. Further, the proportion of segregating families (72.2 %) is close to an independent estimate of Ott's heterogeneity parameter (72.4 %). In summary, this work highlights the ability of HEMIZYG to detect novel, potential risk variants from genotype signal intensity data of related individuals.

Optimizing Measured Genotype Genome-wide Association Analysis for Quantitative Traits in Pedigrees. *J. R. O'Connell* Division of Endocrinology, Diabetes and Nutrition, University of Maryland School of Medicine, Baltimore, MD.

The measured genotype (MG) model is a flexible regression framework for quantitative trait SNP association analysis in pedigree data. The model treats SNP genotypes as fixed effects while controlling for residual familial correlation through a polygenic variance component. The standard genetic models are implemented by coding SNP genotypes as categorical covariates, with natural extensions to multilocus SNPs and haplotypes. Additional covariates such a age, sex, environmental factors, gene-by-gene and gene-environment interactions and population structure are easily incorporated.

The limiting computational step in maximizing the MG likelihood is inverting the variance-covariance matrix, which has order n^3, where n is the dimension of the pedigree kinship matrix. Thus, the computing time to analyze a single SNP quickly increases from seconds to minutes as n increase to from tens to hundreds to thousands, making genome-wide analysis at current SNP chip densities intractable for larger pedigrees. A standard solution is to break up the pedigree into smaller subpedigrees, thus partitioning the variance-covariance matrix into smaller matrices, but at the cost of increased Type I error due to ignored familial correlation.

We present a novel approach based on diagonalizing the variance-covariance matrix to reduce the complexity the likelihood calculation from n^3 to pn^2, where p is the number of covariates. Since the kinship matrix is independent of the SNP, the diagonalization is required only once for the entire genome-wide scan if genotype data is complete, providing significant computational gains. For example, we analyzed 350K SNPs genotyped in 860 Amish subjects within an 8100 pedigree in 70 minutes, compared to an estimated 583 days for SOLAR, a 12,000-fold speed up. To fill in missing genotypes, we have developed an algorithm optimized to compute the posterior probability distribution for untyped subjects.

Our methods have been implemented into user-friendly Open Source software.

Establishment of the pathogenic potential of nonsynonymous variants informs causal relationships in oligogenic disease. *N. Zaghloul[1], J. Gerdes[1], J. Binkley[3], Y. Bromberg[4], Y. Liu[1], L. Davey[1], C. Leitch[1], R. Karchin[2], R. Leibel[5], A. Sidow[3], N. Katsanis[1]* 1) Inst Genetic Medicine, Johns Hopkins Univ, Baltimore, MD; 2) Department of Biomedical Engineering, Johns Hopkins Univ., Baltimore, MD; 3) Departments of Genetics and Pathology, Stanford University Medical Center, Stanford, CA; 4) Columbia University Center for Computational Biology and Bioinformatics (C2B2), New York, NY; 5) Division of Molecular Genetics, Columbia University, New York, NY.

A major challenge in human and medical genetics is the assignment of pathogenicity to alleles, with potentially profound consequences on the interpretation of patient data and management of genetic disorders. This problem is particularly poignant in oligogenic and complex traits, where the phenotype is the result of the combined effect of numerous alleles across several genes. Bardet-Biedl Syndrome represents a useful oligogenic model, where mutations at a second locus have been found in as many as 30% of patients. Numerous mutations in 14 genes have been identified, including 97 missense variants, some of which do not segregate with the disorder in the traditional Mendelian sense. To determine the potential pathogenicity of each known variant and to understand how total mutational load in a functional system modulates penetrance and expressivity, we capitalized on our recent development of in vivo and in vitro assays of ciliary function to assess the functionality of mutant protein. Through an analysis of all missense variants, we show that both loss of function and dominant-negative mutations contribute to the disorder; and a subset of common alleles considered previously to represent benign polymorphisms are in fact detrimental to protein function; in several families, combinations of rare, strong alleles and common, milder alleles, interact to cause the phenotype. Our data represent the first comprehensive modeling of epistatic interactions in human genetic disease and are likely to inform human genetic disorders in which the genotype at a single locus is insufficient to explain phenotypic variability.

Genome-wide analysis of clinical phenotypes in multiple sclerosis (MS). *L. F. Barcellos, International Multiple Sclerosis Genetics Consortium* Div Epidemiology-SPH, Univ California, Berkeley, Berkeley, CA.

Multiple sclerosis (MS) is a chronic inflammatory disorder of the central nervous system (CNS) with a strong genetic component. Variation within the major histocompatibility complex (MHC) region genes on chromosome 6p21, specifically the *HLA-DRB1*15* haplotype, is the strongest genetic risk factor for MS, yet it is estimated to account for only a portion of disease risk. A role for genetic factors influencing clinical outcome in MS is also favorably supported by several lines of evidence. Identification of genetic variants that distinguish particular disease subgroups and/or predict a severe clinical outcome is critical to further our understanding of disease mechanisms and guide development of therapeutic approaches. The Affymetrix GeneChip Human Mapping 500K array was used to examine common genetic variation in 931 MS cases (and trio family members) fully characterized for several clinical phenotypes including age of disease onset, disease subtype, and disease severity. SNPs within two genes (*PHYHIPL* on 10q21 and *CADPS2* on 7q31) were strongly associated with primary progressive MS (PPMS) (OR=3.5, 95% CI: 2.1-5.9; $p=4.1 \times 10^{-7}$, and OR=2.7, 95% CI: 1.7-4.1; $p=4.1 \times 10^{-6}$, respectively). Disease severity was measured using the MS severity score or MSSS (Roxburgh et al. *Neurology* 64:1144-51, 2005) and MS cases were categorized into high (MSSS > 6.0) and low (MSSS < 6.0) groups for comparison. Results showed that SNPs within *RAB40C* and *PIGQ* on 16p13 significantly increased risk for severe disease (OR=1.7, 95% CI: 1.4-2.2; $p=2.1 \times 10^{-6}$) and SNPs within *SDCCAG10* on 5q12 were strong predictors of a more mild disease course (OR=0.6, 95% CI: 0.5- 0.8; $p=8.9 \times 10^{-6}$). Additional genetic variants were also observed to distinguish male and female MS case groups or predict age of symptom onset. The combined results from the very first comprehensive genome-wide association scan of MS clinical outcomes provides an important framework for mapping variants with strong prognostic implications.

PCM1 is recruited to the centrosome by the cooperative action of DISC1 and BBS4 and is mutated in schizophrenia. *N. Katsanis[1], A. Kamyia[2], P. L. Tan[1], K. Kubo[3], C. Engelhard[2], K. Ishizuka[2], A. Kubo[4,5], S. Tsukita[4,5], A. E. Pulver[2], K. Nakajima[3], N. G. Cascella[2], A. Sawa[2]* 1) Inst Genetic Medicine, Johns Hopkins Univ, Baltimore, MD; 2) Departments of Psychiatry and Behavioral Sciences, and Neuroscience, Johns Hopkins University, Baltimore, MD; 3) Department of Anatomy, Keio University School of Medicine, Tokyo, Japan; 4) Department of Cell Biology, School of Health Sciences Faculty of Medicine, Kyoto University; 5) Solution Oriented Research for Science and Technology, Japan Science Technology Corporation, Kyoto, Japan.

Recent genetic studies have suggested that centrosomal dysfunction underlies risk for various neuropsychiatric disorders, because variants in some genes that encode centrosomal proteins have been associated with schizophrenia (SZ) and bipolar disorder (BP). These genes include pericentriolar material 1 (PCM1) and Disrupted-In-Schizophrenia 1 (DISC1). We have reported previously that DISC1, a major susceptibility factor for SZ and BP, plays a crucial role at the centrosome, where it is required for neurite outgrowth and proper development of the cerebral cortex. Therefore, we hypothesized that PCM1, DISC1, and their interacting partners that include the BBS proteins may play a coordinate role in the centrosome and that such interactions might be relevant both to the etiopathology of SZ. Here, we provide biological and genetic evidence that PCM1-DISC1-BBS proteins function in a common centrosomal pathway, associating with SZ. We show that these proteins form a complex at the centrosome through discrete binding domains and that DISC1 and BBS4 act synergistically to recruit PCM1 to the centrosome. Disruption of any of these proteins leads to profound defects in neuronal migration during cortical development, a phenotype accentuated by the coordinate suppression of multiple complex subunits. Finally, we report a pedigree in which a nonsense mutation in PCM1 segregates with SZ spectrum psychosis, suggesting that haploinsufficiency at this locus likely contributes to the development of SZ and highlighting further the critical role of the centrosome in neurodevelopment.

Whole Genome Oligonucleotide Array Comparative Genomic Hybridization (oaCGH) for Clinical Diagnosis: The Line between Call In and Cut Off. *K. Lu[1], B. Xiang[1, 2], M. R. Rossi[1], B. Renu[1], P. Li[1]* 1) Department of Genetics, Yale School of Medicine, New Haven, CT; 2) Department of Human Genetics, University of Miami, Miller School of Medicine, Miami, FL.

Whole genome oaCGH has become the standard of care of most genetic clinics. However, due to the differences in the technical platforms and array designs, there is no consensus as to the analytical validity of these assays. We applied receiver operating characteristic (ROC) statistics to evaluate the sensitivity, specificity, and analytical resolution of oaCGH using Agilents 44K oligonucleotide array (CGH4410B). With specificity preset at 99% and a resolution based on average $\log_2$ ratio of 1, 2, 3, 5 and 7 contiguous probes, the oaCGH showed a sensitivity of 85%, 95%, 96.7%, 98% and 100% for deletion detection and 5%, 40%, 74%, 98% and 99% for duplication detection, respectively. These results indicated that the oaCGH can achieve 99% specificity and 99% sensitivity with an average resolution of 300-500 Kb (5-7 contiguous probes). Using a known deletion in a dilution series of 50%, 33% and 25% mosaicism, the oaCGH showed 85% sensitivity and 95% specificity in detecting 50% mosaicism; however, increased test-to-test variations and reduced sensitivity were noted as the mosaic percentage decreased. To further enhance the diagnostic accuracy, sex-matched alternate referred dye-swap (SMARDS) design was introduced. Each sample was labeled as test by Cy5 and as reference by Cy3 in alternate pairs, allowing for dye-swap analysis by joining the test and reference readouts. The SMARDS method has increased the average analytical resolution to 150-250 Kb (3 contiguous probes in a dye-swap pattern) and detected significantly ($p<0.001$) more copy number variants (CNVs) (CNVs/case: 1.4921.286, n=242) than the widely used method of single test labeling and pooled controls (0.4690.683, n=175). FISH analysis using targeted BAC clones was performed to confirm these genomic imbalances and to assess suspected mosaic patterns. These data suggest that the 44K oaCGH could make correct calls for genomic imbalances in a resolution of >150 Kb (3-7 contiguous probes) with 99% sensitivity and 99% specificity.

Variation near Complement Factor I is associated with risk of Advanced Age-Related Macular Degeneration. *J. A. Fagerness[1,2], J. B. Maller[1,2,4], B. M. Neale[1,2,3], R. C. Reynolds[5], M. J. Daly[1,2], J. M. Seddon[5]* 1) CHGR, Massachusetts General Hospital, Boston, MA; 2) Program in Medical and Population Genetics, Broad Institute of Harvard and MIT; 3) SGDP Centre, Institute of Psychiatry, Kings College London; 4) Department of Statistics, University of Oxford, Oxford UK; 5) Ophthalmic Epidemiology and Genetics Service, New England Eye Center, Tufts-Medical Center.

Age-related macular degeneration (AMD) is the leading cause of irreversible blindness in older adults and has become a model for the genetics of complex disease. AMD has been shown to be associated with variants on chromosome 1 (CFH), chromosome 6 (CFB; C2), chromosome 10 (LOC387715/ARMS2), and chromosome 19 (C3) and has clearly identified the primary role of the complement pathway in disease pathogenesis. We tested 29 SNPs across a region spanning complement factor I (CFI) and phospholipase A(2) Group 12A (PLA2G12A) for association with AMD in 1228 cases of both neovascular (wet) and geographic atrophy (dry) advanced AMD and 825 age and sex matched controls. Conditioning on our most associated SNP, rs10033900 (p-value = 6.46×10^{-8} with an OR = 0.7056), we observed no further significant independent associations. We did, however, observe modest residual association at two neighboring, highly correlated SNPs. This result suggests that rs10033900 may not be the causal variant but may be highly correlated with said variant. Therefore, we applied multi-marker haplotype tests in an attempt to refine and isolate the association signal. We tested the two-marker haplotype of the two closest SNPs to rs10033900, both 5 (rs13117504) and 3 (rs11726949). The two-marker haplotype between rs13117504 and rs10033900 shows a somewhat stronger association to AMD than either SNP alone with a p-value = 1.18×10^{-8}. All exons were sequenced across the block of linkage disequilibrium (LD) where the associated SNPs were found. This block of LD contained all four exons of PLA2G12A and the last two exons of CFI, but none of the variation discovered could explain the observed association.

Assessing Response of Hunter Syndrome to Idursulfase (Elaprase) Enzyme Replacement Therapy. *B. Najafian, A. Van Heest, C. Whitley* University of Minnesota, Minneapolis, MN.

We examined the outcome of the severe form of Hunter syndrome after treatment with idursulfatase (Elaprase) enzyme replacement therapy (ERT). Proband C presented in October, 2005 with a murmur and was referred to a geneticist. High urine glycosaminoglycan (GAG) was found. When several lysosomal enzymes were reported normal, it was concluded that C did not have a mucopolysaccharidosis condition. The diagnosis was made in May, 2006 at 5 years of age when the father reviewed the medical record, and challenged the specialists conclusions. With additional testing, C was found to have deficient iduronate-2-sulfatase (IDS) activity. This motivated the parents to relocate for ongoing care. Upon presentation to our clinic, C was having episodic chest pain thought by his parents to be life-threatening heart disease. Gastro-esophageal reflux was found, and responded dramatically to medical management. Molecular analysis identified the 1449delA mutation of the IDS gene associated with severe neuropathologic Hunter syndrome. Brother Ms diagnosis was made in May, 2006. After starting on idursulfase ERT urine GAG levels declined, neuropsychometric scores remained unchanged, but orthopedic problems have progressed. Carpal tunnel syndrome and finger contractures have motivated surgical procedures. Both have hearing loss, hand and finger contractures, and umbilical hernia. The excellent learning progress of these children led the parents to ask two important questions: (1) Does reduction of urine GAG reflect a systemic halt, or reversal of pathology? (2) With a systemic response, what is the long-term neurologic prognosis for these children with severe mutations associated with mental retardation? Close monitoring is being done to answer these questions: Tenosynovial and liver biopsies were performed before idursulfase therapy was started. Electron microscopy showed lysosomal inclusions in fibroblasts of tenosynovium, but not in the liver. Quantitation of these inclusions using unbiased stereology will be used to assess possible reversal of pathology in the follow-up biopsies.

RPGRIP1L interacts biochemically with a diverse ciliary protein complex and contributes causal and modifying alleles across all ciliopathies. *E. E. Davis[1], H. Khanna[2], A. Estrada[2], Z. M. Zamalloa[2], I. Lopez[3], A. I. den Hollander[4], M. N. Zonneveld[4], L. M. Davey[1], M. I. Othman[2], N. Waseem[5], C. Maubaret[5], I. MacDonald[6], D. M. Muzny[7], T. Attie-Bitach[8], C. A. Johnson[9], R. K. Koenekoop[3], F. Hildebrandt[10], R. A. Gibbs[7], A. Swaroop[2,10,11], N. Katsanis[1]* 1) Inst Genetic Medicine, Johns Hopkins Univ, Baltimore, MD; 2) Department of Ophthalmology and Visual Sciences, University of Michigan, Ann Arbor, Michigan; 3) McGill Ocular Genetics Laboratory, McGill University, Canada; 4) Department of Human Genetics, Nijmegen Centre for Molecular Life Sciences, The Netherlands; 5) Institute of Ophthalmology, UCL, London, UK; 6) Ophthalmic Genetics and Visual Function Branch, National Eye Institute, USA; 7) Human Genome Sequencing Center, Baylor College of Medicine, USA; 8) Département de Génétique, Paris Cedex 15, France; 9) Section of Ophthalmology and Neurosciences, Leeds Institute of Molecular Medicine, United Kingdom; 10) Department of Human Genetics, University of Michigan, USA; 11) National Eye Institute, Bethesda, Maryland, USA.

Loss of function mutations in RPGRIP1L, a ciliary protein, cause Meckel-Gruber (MKS) and Joubert (JBTS) Syndromes, two traits at the extreme severity of the ciliopathy spectrum. Here we demonstrate that RPGRIP1L interacts directly with RPGR, encoded by the locus mutated most frequently in RP, as well as with a multitude of BBS and NPHP proteins. Prompted by these data, we interrogated the potential mutational load of RPGRIP1L in a ciliopathy cohort comprised of >500 patients spanning the phenotypic spectrum of severity, including LCA, Senior-Loken Syndrome (SLS), NPHP, BBS, JBTS, and MKS. We identified a large allelic series of missense variants, which occur in concert with null genetic lesions at other ciliary protein encoding loci. We also demonstrate that these variants either attenuate or completely abrogate the biochemical interaction between RPGRIP1L and RPGR, data substantiated further by in vivo testing of each allele in a zebrafish ciliopathy model. Taken together, our data suggest that RPGRIP1L is a pan-ciliopathy gene and highlight the emerging utility for medical resequencing in dissecting total mutational load.

Van Allen-Myhre Syndrome and Goltz Syndrome are allelic. Report of two cases with PORCN gene mutations.

S. Parkash[1,2], S. Keating[3], I. Van den Veyver[4], K. Siriwardena[5], E. Kolomietz[2], A. Toi[6], C. Zaitsoff[7], D. Purvis[8], A. Patel[4], X. Wang[4], D. Chitayat[1, 2] 1) Dept Clinical/Metabolic Gen, Hosp Sick Children, Toronto, ON, Canada; 2) Prenatal Diagnosis and Medical Genetics Program, Mount Sinai Hospital, Toronto, Canada; 3) Department of Laboratory Medicine and Pathobiology, Mount Sinai Hospital, Toronto, Canada; 4) Department of Obstetrics and Gynecology and Molecular and Human Genetics, Baylor College of Medicine, Houston, USA; 5) Clinical Genetics NZ, Auckland, New Zealand; 6) Department of Diagnostic Imaging, Mount Sinai Hospital, Toronto, Canada; 7) Northern Regional Genetic Service, Auckland Hospital, New Zealand; 8) Department of Dermatology, Auckland City Hospital, New Zealand.

Van Allen-Myhre Syndrome (VAMS) is a rare condition consisting of ectopia cordis, omphalocele, ectrodactyly, absent sacrum, radial hypoplasia, microphthalmia, hemifacial microsomia and cutis aplasia, among other findings (Van Allen and Myhre, 1991). Some of these features overlap with findings in Goltz syndrome (GS), which suggest that they are allelic. The finding that GS is caused by mutations in the PORCN gene enabled us to test this possibility. We report two cases with VAMS, one of which supports the assumption that VAM and GS are allelic. Case 1 - A fetus with absent and possibly bifid sternum, omphalocele, hyperlordosis, scoliosis, and bilateral ectrodactyly consistent with VAMS. Karyotype and microarray analysis were normal, with no deletion in the Xp22 region. A deletion in the PORCN gene was detected by qPCR. Case 2 - A male with an antenatal diagnosis of Klinefelter syndrome, bladder extrophy, ectrodactyly, symmetrical IUGR, hypoplastic nails, linear skin lesions, right hemifacial microsomia, and bilateral microphthalmia consistent with VAMS. No mutation in the PORCN gene was found. The finding of mutations in the PORCN gene in our patients supports the assumption that VAMS is the same condition as GS.

The power of the allele-based N-Test in linkage analysis. *S. Khan[1, 3], S. Shah[3], D. E. Weeks[1,2]* 1) Department of Human Genetics, University of Pittsburgh, Pittsburgh, PA 15261, USA; 2) Department of Biostatistics, University of Pittsburgh, Pittsburgh, PA 15261, USA; 3) Department of Statistics, University of Peshawar, Peshawar, NWFP, Pakistan.

There are many tests of inheritance based upon sibling information for diseases that have late onset. The N-test (Green et al. 1983) is one of these tests, which utilizes information from affected siblings. The N-test is the count in affected siblings of the most frequently occurring haplotype from the father plus the analogous count from the mother. When applied to haplotypes, the N-test excludes recombinant families from the analysis. In this study we modified the N-test to be based on alleles instead of haplotypes. This modified allele-based N-test can include all families (recombinant as well as non-recombinant). We carried out a simulation study to compare the power of the allele-based N-test with the powers of the Sall and Spairs non-parametric statistics as computed by Merlin. The powers of the allele-based N-test, Sall and Spairs statistics are identical to each other for affected sibships of size 2 and 3. For affected sibships of larger sizes, the powers of the Sall and Spairs statistics are larger than the power of allele-based N-test. These simulation-based results are consistent with earlier results based on analytical computations.

GRM7 variants confer susceptibility to age-related hearing impairment. *J. Ohmen[1], R. Friedman[1], L. Van Laer[2], M. Huentelman[3], E. Van Eyken[2], J. Corneveaux[3], W. Tembe[3], S. Thys[2], E. Fransen[2], J. Huyghe[2], J. Pearson[3], D. Craig[3], D. Stephan[3], G. Van Camp[2], additional member of the European Presbycusis Genetic Study* 1) House Ear Institute, Gonda Research Center for Cell and Molecular Biology, Los Angeles, California, USA, 90057; 2) Department of Medical Genetics, University of Antwerp, Antwerp, Belgium, B-2610; 3) The Translational Genomics Research Institute, Phoenix, Arizona, USA, 80054.

Age-Related Hearing Impairment (ARHI) is a complex disease resulting from the interaction between environmental and genetic factors and is the most prevalent sensory impairment in the elderly. While the environmental factors conferring altered risk for ARHI have been extensively studied, the genetic risk factors are unknown. Here we describe the results of a whole genome association study with 1,692 ARHI samples. Using an age- and sex-independent measure of hearing loss, the samples from individual centers were combined into good and bad hearing pools and assayed in triplicate on the Affymetrix 500K GeneChip. The highest ranked SNPs identified in the initial pooling experiment were confirmed by genotyping individual samples in the primary cohort. Subsequently, the 23 most interesting SNPs were individually genotyped in a second cohort with the same phenotypic criteria. This resulted in the identification of a highly significant replicated SNP located in GRM7, the gene encoding the type 7 metabotropic glutamate receptor (mGluR7). We performed functional studies and show that mGluR7 is expressed in the inner ear hair cells and in the spiral ganglion nerve cell bodies of the inner ear. Together these data indicate that common alleles of GRM7 contribute to an individuals risk of developing ARHI and suggest a possible functional role for mGluR7 in hair and spiral ganglion cells of the ear. Our central hypothesis is that common variation within GRM7 gene may lead to altered levels of GRM7 protein in the afferent auditory synapse, resulting in perturbations of glutamate metabolism.

Building, mining, and managing array CGH databases define a new paradigm for the identification of clinically significant cytogenetic aberrations. *B. C. Ballif, J. Coppinger, B. A. Torchia, B. A. Bejjani, L. G. Shaffer* Signature Genomic Laboratories LLC, Spokane, WA.

The use of microarray-based copy-number screening methods such as array CGH have revolutionized cytogenetic diagnostics by facilitating the identification of previously unrecognized chromosomal syndromes, refining critical regions for established genetic disorders, and broadening our view of the normal diploid genome. However, these high-resolution whole genome screens generate a wealth of genomic information which can be difficult to interpret in a clinical cytogenetics setting. Indeed, the prevalence of copy-number variants of unclear clinical significance underscores the need for the development of readily accessible diagnostic tools and databases of documented chromosome abnormalities to distinguish between benign copy-number variants and those of clear clinical significance. The utility of building, mining and managing an array CGH database is illustrated through the identification of three rare chromosome abnormalities that may not have been recognized otherwise. First, we have refined the microcephaly locus on 1q44 by characterizing the deletion sizes of three patients with interstitial deletions surrounding the *AKT3* gene. Second, we report the identification of three additional cases of microdeletions encompassing *TCF4* on 18q21.2 recently reported to result in Pitt-Hopkins syndrome. The identification of these patients will aid in the further delineation of the phenotype associated with this deletion. Third, we have identified a previously unrecognized putative microdeletion syndrome on 17q23.2. This region is flanked by low-copy repeats and may be a recurrent novel microdeletion syndrome. We anticipate that array CGH databases will have a profound impact on our ability to understand the clinical significance of chromosome abnormalities identified by high-resolution copy-number analysis.

Do Biobank Regulations Parallel Recommendations and Guidelines Presented in the Literature? *I. T. Gordon, T. Caulfield* Health Law Institute, University of Alberta, Edmonton, Alberta, Canada.

Large genetic repositories, often referred to as biobanks, present an important platform for genetic research. Many scientists believe the genetic study of large population cohorts is the next logical step towards understanding and treating a variety of diseases. It is therefore unsurprising that large, often national, biobanking projects are already underway in countries such as the United Kingdom, Canada, Latvia, Estonia, Iceland and are being considered by others. While these biobanks may be a welcome aide to researchers, they are not without their difficulties. Indeed, a number of important issues must be dealt with by the governance structures seeking to establish these databases. This study investigates how recent major policy documents address five of the major governance issues facing biobanks. These issues include the informed consent of participants, the right of participants to withdraw from the study, how the database will protect the privacy of participants including who will be allowed access to the database, the ownership of samples and study results as well as the need for an independent ethics review board in governing the biobank. This comparative analysis looks for consensus and divergence of views within major policy documents and compares them to themes in the recent, relevant, academic literature. The study found a variety of common themes, particularly around the need for governance, consent and the right to withdraw. However, a comparison with the academic literature reveals that broad, principled based, consensus has yet to be achieved.

The spectrum of BCSIL related conditions. *N. Longo[1], M.-A. Abbott[4], A. Yatsenko[2], D. Dimmock[3], L.-J. C. Wong[2]* 1) Dept Peds, Div Med Genetics, Univ Utah, Salt Lake City, UT; 2) Molecular and Human Genetics, Baylor College of Medicine, Houston Texas; 3) Pediatrics, Medical College of Wisconsin, Milwaukee, WI; 4) Pediatric Genetics, Baystate Medical Center, Springfield,MA.

Background Specific autosomal recessive mutations in *BCS1L* have recently been described to cause GRACILE syndrome. This is a disorder of people of Finnish heritage associated with a prenatal onset disease of Growth Retardation, Aminoaciduria, hepatic Cholestasis, Iron overload and Lactic acidosis and Early death. It is not associated with classical hair changes. Similar presentations have been described in 3 British kindreds with the addition of mitochondrial complex 3 deficiency. Turkish patients with mutations in *BCS1L* have a slightly different phenotype of tubulopathy, encephalopathy, and liver failure due to complex III deficiency. Bjornstad syndrome is a clinically distinct disorder of Pili torti, and deafness, it is caused by milder mutations in *BCS1L* and is not associated with complex 3 deficiency **Cases** The 1st patient failed her newborn hearing screen but otherwise had a normal early infancy. At 3m of age she started to failure to thrive with poor feeding, by 6m of age she had significant developmental delay. At this point she had a normal brain MRI but significant lactic acidosis. At 7m of age, when referred to genetics, she had sparse abnormal scalp hair, mild liver dysfunction and renal Fanconi syndrome. Subsequently her MRI developed changes suggestive of Leigh syndrome. ETC showed profound deficiency of complex III, sequencing revealed 2 mutations in *BCS1L*. The 2nd case presented with Infantile spasms at 6m of age. She was noted at this point to have significant failure to thrive, renal Fanconi syndrome, Cholestatic jaundice and lactic acidosis. She died at 7 months of age. Postmortem ETC studies revealed profound complex III deficiency and sequencing revealed mutations in *BCS1L*. **Conclusions** These reports suggest that Bjornstad syndrome and GRACILE are ends of a phenotypic spectrum and not discrete disorders. The also suggest that mutations in *BCS1L* should be considered in children with renal Fanconi syndrome especially when associated with hearing loss or hepatic disease.

MicroRNA-Mediated Cardiac Dysfunction and Myosin Regulation. *J. T. C. Shieh[1], D. Srivastava[2]* 1) University of California San Francisco; 2) Gladstone Institute for Cardiovascular Disease, San Francisco, CA.

Background: Heart failure is common in patients with congenital heart disease and with cardiomyopathies, yet our understanding of the basis of disease is limited to a few genes. Since gene dysregulation can lead to cardiac failure, we hypothesized that microRNAs, small non-coding RNAs that regulate other genes, could be important in disease. **Purpose:** We sought to identify novel, tissue specific microRNAs from the human heart and determine their role in cardiac function using model organisms. **Methods:** We performed a screen of over 295 microRNAs to detect those expressed preferentially in the human heart using microRNA microarrays. Cardiac-specific microRNA expression was confirmed using qPCR and Northern blotting on human and mouse tissues. To mimic states where microRNAs are elevated, we generated cardiac-specific transgenic mice to overexpress the microRNA. We assessed cardiac function in transgenic mice using echocardiogram and performed histology. In vitro microRNA-mRNA experiments were used to establish potential gene expression pathways regulated by the microRNA. **Results:** We found a previously uncharacterized microRNA that demonstrates strong expression in human heart. This non-coding RNA is sufficient to cause cardiomyopathy in mice, and the conservation in the microRNA sequence from fish to humans supports a key gene regulatory role. Interestingly, myogenesis is accompanied by increased levels of the microRNA while inhibition of the microRNA in vitro reduces myosin expression, suggesting a direct role in regulating muscle. When we overexpressed the microRNA in mouse hearts, we found myosin upregulation in transgenic lines. Interestingly, microRNA-overexpressing mice developed cardiomyopathy. We have identified a potential mechanism of disease since the microRNA appears to affect target mRNAs important in myosin regulation. **Conclusions:** These studies demonstrate that expression of a single microRNA is sufficient to cause cardiomyopathy and suggest the importance of microRNA gene regulation during cardiac disease. These data suggest the need for more widespread analysis of non-coding transcripts in unexplained cardiac disorders.

Germline missense mutations in a DNA glycosylase gene, NEIL2 is associated with lung cancer. *A. Maiti[1], M. Hegde[1], G. Wang[3], N. He[1], D. Bannerjee[1], X. Xie[1], Y. Lee[3], B. Shen[2], S. Mitra[1], T. Hazra[1]* 1) Dept Biochem & Molecular Biol, Univ Texas Medical Branch, Galveston, TX.USA; 2) City of Hope Medical Center, California, USA; 3) Chinese Academy of Sciences, Beijing, China.

Genetic predispositions along with environmental factors including genotoxic agents have critical etiologic roles in developing lung cancers and a handful of genes are identified for lung cancer. We identified several missense mutations in the coding regions of a DNA glycosylase gene, NEIL2 and studied their association with lung cell carcinoma. Analyzing 170 lung cancer patients with equal number of healthy control, we identified 4 misense mutations. Among them two are rare polymorphism and in an extended study with large population size, other two shows ethnic specific associations. Two SNPs are associated in caucasian lung cancer patients (rs8191664 p=0.028, OR=3.42, 95% CI 1.12-10.2, n=case:control-112:188) and (rs8191613, p=0.008, OR=5.76, 95%CI 1.15-21.4, n=case:control=112:188). In chinese population, rs8191664 (OR= 1.31, 95%CI 1.03,1.66 p=0.002, n=case : control=670:666) is moderately associated. It appears that risk factor for lung cancer of rs8191664 is much more higher in caucasian population than chinese population. A large number of caucasian patient analysis is in progress. However, purified mutant proteins for these SNPs have lower glycosylase activities, lower overall repair activities in compare to wild type NEIL2 protein in vitro. These mutant proteins also show less interaction with DNA polymerase and ligase3, prerequisites for DNA repair activity in vivo. These results suggest that these SNPs may impair DNA repair activity in vivo and contribute to the development of lung cell carcinoma.

High Reslolution microarray analysis of CpG island methylation. *D. Roberts[1], A. Wong[1], R. Straussman[2], B. Curry[1], Z. Yakhini[1], I. Steinfield[2], A. Ashutosh[1], R. Saxena[1], H. Cedar[2], D. Roberts[1]* 1) Dept Research & Development, Agilent Technologies, Santa Clara, CA; 2) Dept. of Cellular Biochemistry and Human Genetics, Hebrew University, Jerusalem, Israel.

CpG islands are stretches of high GC content DNA containing multiple CpG dinucleotides. When CpG dinucleotides within these islands are methylated, especially in promoter regions, expression of the corresponding downstream genes is most often repressed. Aberrant CpG island methylation is implicated in genetic diseases and especially cancer. We have developed a microarray based solution for determination of methylated and unmethylated CpG islands in the human genome. This microarray contains ~200,000 oligo probes tiling the 21 megabases of ~25,000 unique CpG islands, with an average spacing between probes of ~95 base pairs. Methylated DNA is immunoprecipitated (mDIP), fluorescently labeled, and competitively hybridized to the array against differentially labeled pre-immunopreciptiated DNA. We have developed a methylation calling algorithm to accurately determine methylated and unmethylated regions. We describe the application of this method and algorithm in a normal adult liver DNA model system. We demonstrate the ability of the assay to discriminate between regions confirmed to be methylated or unmethylated by bisulfite sequencing. We then apply the whole-genome assay to colon and colon carcinoma tissue DNA. We describe differential methylation between normal and cancer tissues.

Array CGH detects tetrasomy 12p in blood from Pallister-Killian syndrome cases without invasive skin biopsy. *J. A. Rosenfeld, B. A. Torchia, B. C. Ballif, L. G. Shaffer* Signature Genomic Laboratories, Spokane, WA.

Pallister-Killian syndrome (PKS) is a genetic disorder characterized by mental retardation, seizures, streaks of hypo- or hyperpigmentation and dysmorphic features. PKS is associated with tissue-limited mosaic tetrasomy 12p, usually caused by an isochromosome 12p. The mosaicism is usually detected in cultured skin fibroblasts or amniotic cells and rarely in PHA stimulated lymphocytes, which suggests stimulation of T-lymphocytes may distort the percentage of abnormal cells. We recently reported the identification by microarray-based comparative genomic hybridization (aCGH) of a previously unsuspected case of tetrasomy 12p caused by an isochromosome 12p. Here we report six additional individuals with tetrasomy 12p characterized by our laboratory. All six individuals were referred for mental retardation/developmental delay and/or dysmorphic features. In each case, aCGH using genomic DNA extracted from whole peripheral blood detected copy-number gain for all clones for the short arm of chromosome 12. In all but one case, FISH on metaphases from cultured lymphocytes did not detect the copy-number gain; in the remaining case, metaphase FISH on cultured lymphocytes showed an isochromosome in 10% of cells. However, interphase FISH using probes to 12p on peripheral blood smears showed additional signals in 28-50% of cells. Microarray and FISH analysis on cultured skin biopsies from two individuals confirmed the presence of an isochromosome 12p. Our results demonstrate aCGH using genomic DNA from whole peripheral blood can detect chromosome abnormalities that are not present in stimulated blood cultures and would otherwise require invasive skin biopsies for identification.

Metabolic syndrome gene network and its association with factors of metabolic syndrome. *A. T. Kraja[1], Y. M. Park[2], I. B. Borecki[1], H. K. Tiwari[3], J. S. Pankow[4], S. C. Hunt[5], U. Broeckel[6], D. K. Arnett[7], D. C. Rao[2], M. A. Province[1]* 1) Div. Statist. Genomics, Washington U., MO; 2) Div. of Biostat. Washington U., MO; 3) Dep. of Biostat. U. of Alabama at Birmingham; 4) Dep. of Epi, U. of Minnesota; 5) Cardiovasc. Gen. U of Utah; 6) Hum. Mol. Gen. Ct., Med. Coll. of Wisconsin; 7) Dep. of Epi, U. of Alabama at Birmingham.

We performed an extended review of the curated literature to identify genes that influence metabolic syndrome (MetS). From 1,188 unique genes that contributed to 1 or more MetS domains, 123 genes were identified as candidate contributors to MetS because each was connected to 3 or more domains. The domains selected were obesity, dyslipidemia, glucose intolerance, insulin resistance, and high blood pressure. As a result of these gene findings, a gene network of 123 genes was built where 60 genes connect to 3 domains, 39 others to 4 domains, and 24 to 5 domains. Of these candidate genes, 40 were also classified as candidate contributors to inflammation. We tested association of SNPs across these candidate genes with each of the 4 MetS-factor-domain scores. This examination was based on factor analysis on 11 MetS risk factors using 1,470 white subjects of the Hypertension Genetic Epidemiology Network Study. From the list of 123 genes, 63 of them had at least one SNP associated significantly with the MetS factor scores. The most SNPs associated with one or more MetS-factor-domains belonged to genes ABCA1 (14 SNPs), ALMS1 (14), CYP19A1 (9), FTO (24), LIPC (21), and SELE (9 SNPs). We are currently carrying out replication of the significant associations in the Family Heart Study involving ~1,000 subjects with measurements at two time points. The MetS gene network helped to indentify hypothesis-driven focused associations to better understand the MetS genetic mechanisms. Such an approach has the advantage of protection from multiple testing problems.

Single Nucleotide Polymorphisms of *TNF, TNFR1* and *TNFR2* genes in Mexican lepromatous leprosy patients.

M. Montoya-Buelna[1], M. Fafutis-Morris[2], JF. Muñoz-Valle[3], LE. Figuera-Villanueva[1] 1) CIBO, Instituto Mexicano del Seguro Social, Guadalajara, Jalisco, México; 2) Centro de Investigación en Inmunología y Dermatología. Guadalajara, Jalisco, México; 3) Instituto de Investigación en Reumatología y del Sistema Músculo Esquelético. Guadalajara, Jalisco, México.

Leprosy is a chronic infectious disease caused by *Mycobacterium leprae*, where tumor necrosis factor-alpha (TNF-) activates several effector mechanisms against this infection binding to its receptors (TNFR1 and TNFR2). The TNF- promoter polymorphism at position -308 (named TNF2) has been extensively associated with a variety of autoimmune and infectious diseases, such as leprosy. In addition, polymorphic variants in both TNFR genes are associated with several disorders, although in leprosy patients have not been yet explored. OBJECTIVES: To analyze the association between SNPs at positions -308 in TNF, -383 TNFR1 and +196 TNFR2 in Mexican leprosy patients. DESIGN AND METHODS: Genomic DNA was extracted from peripheral blood cells from 42 lepromatous leprosy (LL) and 5 tuberculoid leprosy (TT) patients. As a control group, 42 healthy subjects (HS) were included. TNF-, TNFR1 and TNFR2 gene polymorphisms were analyzed by PCR and restriction fragment length polymorphism (PCR-RFLP). RESULTS: TNFR2 196 R/M polymorphism was statistically different ($p<0.05$) in the genotypes frequencies between LL and TT patients which lead to increase the susceptibility to develop TT leprosy. TNFR2 196R allele, TNF -308 G/A and -383 TNFR1A/C polymorphisms were not statistically different when compared to LL, TT and HS groups. CONCLUSIONS: Our study suggests that TNFR2 196 R/M genotype is associated with the susceptibility to develop TT leprosy. Polymorphisms in TNF promoter region and TNFR1 receptor genes are not associated with any pole in Mexican leprosy patients. Further studies are needed to elucidate the role of other polymorphisms that increase susceptibility to develop some leprosy pole.

Bayesian inference of fine-scale recombination rates and hotspots using population genomic data. *Y. Wang, B. Rannala* Genome Center and Department of Evolution and Ecology, University of California Davis.

Inferring how recombination rates vary and how recombination hotspots are distributed across genomes is a fundamental problem in population genetics. As more large-scale human genomic data become available, a picture of how recombination rates vary on a genome-wide scale can be inferred using statistical models. Recently, several statistical methods for estimating fine-scale recombination rates using population samples have been developed. However, currently available methods that can be applied to large-scale data are limited to approximated-likelihoods. We developed a full-likelihood Markov Chain Monte Carlo (MCMC) method for estimating recombination rate under a Bayesian framework. In our method, genealogies underlying a sampling of chromosomes are effectively modeled by using marginal individual SNP genealogies related through an ancestral recombination graph. Based on the observed patterns of recombination rates and hotspots obtained from sperm-typing studies, we designed a new model for the distribution of background recombination rates and hotspots. A geometric Brownian motion model is used to account for the random changes in background recombination rates. A Markov process was used to model the distribution of the intervals between hotspots and the duration of hotspots. A reversible jump MCMC scheme is used to estimate the posterior distributions of parameters of interest. The posterior probability that a chromosomal interval contains a hotspot is inferred and plotted along a chromosome. Simulation studies show that the method performs well, and if the hotspot signal from a locus is strongly supported by the data, it is highly likely that the locus is on a hotspot. The method was applied to two human population genetic variation data sets that have been studied previously by sperm-typing. Our results are consistent with estimated hotspot locations from sperm crossover analysis. We also applied our method to a population genetic data set of SNPs spanning chromosome 19 that were sampled from three populations. The biological properties of chromosomal regions with a high probability of containing hotspots were investigated.

Prevalence of Consanguinity in Yazd Province of Iran and It's Role in RPL. *S. Seyedhassani[1,2], A. Aflatoonian[1], N. Tabibnejad[1], G. Modabber[2], R. Mirfakhrai[2], S. Kalantar[1], M. Houshmand[2]* 1) Medical Genetics Dept, Res/Clinical Ctr Infertility, Yazd, Iran; 2) National Institute of Genetic Engineering and Biotechnology, Tehran, Iran.

Introduction: A consanguineous relationship is one between blood relatives who have at least one common ancestor no more remote than a great-great grandparent. While relatively rare in western populations, consanguinity is common in many populations of the world. pregnancy loss is the most common complication of pregnancy. About 1% to 2% of couples experience three or more consecutive spontaneous pregnancy loss, suggesting some underlying operative mechanisms. Many studies have shown that among the offspring of consanguineous marriages there is an increased incidence of both congenital malformations and other conditions which will present in fetal life and later. Material and methods: We studied 5200 married defined couples in 260 randomized clusters that were divided in ten different rural and urban areas. These couples were interviewed based on using a structured questionnaire to ascertain the prevalence of consanguinity and related epidemiological findings. On the other hand, we assess the consanguinity in 261 couples were involved with repeated pregnancy loss. Results: There were consanguineous marriages in 2153 cases (41.1%)(CI 95% from 41.2% to 42.7%) in different degrees including degree 2 (0.04%), degree 3(27.3%), degree 4 (7.4%), degree 5(6.7%). Thus, 58.9% of population had nonfamilial or more remote than five degree marriages. There were 261 cases of RPL that were encountered and referred to our center. In these couples 142 had consanguineous marriage (54.4%) in degree2 (2.3%), degree3 (37.5%), degree4 (7.7%), degree5 (6.9%). Conclusion: In Iran, there was an incidence of high consanguineous marriage near to neighboring countries such as Pakistan, Kuwait and Saudi Arabia. Consanguinity was related to in expression of RPL (p 0.00 and Odds Ratio= 1.69, 1.31< OR< 2.18). These findings can implicate the probability of bilateral recessive genetic effects leading to repeated abortion in encountered couples and thus, avoidance of familial marriage is suggested .

Epigenetic mechanisms and autoimmunity: a pilot study testing DNA methylation profiles in multiple sclerosis cases and controls. *F. B. S. Briggs[1], S. Clark[1], J. Oksenberg[2], C. Schaefer[3], L. F. Barcellos[1,3]* 1) Univ of California, Berkeley, CA; 2) Univ of California, San Francisco, CA; 3) Div of Research, Kaiser Permanente, CA.

Multiple sclerosis (MS), a chronic inflammatory autoimmune disease (AD) of the central nervous system, is characterized by incomplete concordance in monozygotic twins, female preponderance, the presence of other co-morbid autoimmune conditions, and variable patterns of symptoms and disease progression. A large number of MS studies have consistently shown strong associations with *HLA-DRB1*15*; however, the identification of additional disease loci is critical to fully understand pathogenesis. Previous studies support a role for aberrant DNA methylation (heritable and stable methylation of DNA CpG loci that alters gene expression) in autoimmunity. To identify novel epigenetic loci in MS, we conducted a pilot study testing the DNA methylation patterns of 807 candidate genes (1,505 CpG loci) in 109 Caucasian female MS cases and 61 female controls. Two-sided t-tests were used to compare methylation profiles at each locus in a two-stage approach which allowed for replication of promising results, (Stage I: MS=47, controls=36; Stage II: MS=62, controls=25). We identified a CpG locus within *TJP2* as being significantly unmethylated in MS cases (p1=0.03; p2=0.007) when compared to controls. When analyses were restricted to primary progressive MS (PPMS) cases only (PPMS=24; controls=25), the *TJP2* locus remained the most significant result (p=0.001). We also compared cases with particular co-morbid AD conditions known to cluster with MS (Hashimotos thyroiditis, inflammatory bowel disease and psoriasis; Stage I: MS/AD=22, controls=36; Stage II: MS/AD=29, controls=25). A total of 16 genes emerged as differentially methylated in cases when compared to controls ($p1<0.05$; $p2<0.05$). The two most promising candidates were *HLA-DOA*, which participates in the presentation of peptides bound to MHC class II molecules, and *CDK6*, which is involved in CD8 memory T cell division. Several potential epigenetic candidates that may contribute to MS etiology were identified. Further research in this important area is needed.

Genome-Tissue Expression Analysis (GTEx), Digital Transcript Expression (DTE) and Genomic Congruence Analysis of the Cerebellar Cortex in Schizophrenia: A Model for Analysis of Complex Traits with Massively Parallel mRNA Sequencing. *S. Kingsmore[1], J. Mudge[1], N. Miller[1], G. May[1], J. Huntley[1], A. Farmer[1], R. Langley[1], J. van Velkingburgh[1], D. Gessler[1], G. Schroth[2], I. Khrebtukova[2], S. Luo[2], L. Zhang[2], M. Garcia[3], R. Harlan[3], S. Khalsa[3], R. Wolfinger[4], S. Martin[4], R. Roberts[5], N. Perrone-Bizzozero[6]* 1) Natl Ctr Genome Resources, Santa Fe, NM; 2) Illumina Inc., Hayward, CA; 3) Northern New Mexico College, Española, NM; 4) SAS Institute, Cary, NC; 5) Department of Psychiatry, University of Alabama at Birmingham, AL; 6) Department of Neurosciences, University of New Mexico, Albuquerque, NM.

Purpose: Massively parallel mRNA sequencing was performed to assist in characterization of a prototypic complex trait, schizophrenia (SCZD). Methods: 16.7 GB of mRNA sequence was generated from 20 cerebellar cortices (14 with SCZD and 6 controls) using Illumina sequencing-by-synthesis. Sequences were aligned to reference databases, gene abundances were determined by aligned read frequency and nucleotide variants identified using trained bioinformatic filters. Gene expression differences were identified by analysis of variance; cis-acting eSNPs were identified by Infinium genotyping of samples and assessment of significant deviations in read-count-based allele frequency at heterozygote loci; Variant associations with SCZD were identified. Results: Of 33,200±1,000 expressed transcripts, 204 differed significantly between SCZD and controls. GO annotation showed significant SCZD associations with Golgi apparatus and vesicle-mediated transport. DTE was more sensitive and accurate than array hybridization. 10,022 genes contained novel splice isoforms. Of 453,555 single nucleotide variants identified, 56 were eSNPs and 113 showed significant associations with SCZD, including rs4894 in ATF4, a DISC1-interactant that maps within an SCZD QTL. Conclusion: Massively parallel mRNA sequencing appears to have utility for Genome-Tissue Expression Analysis, Digital Transcript Expression and Genomic Congruence Analysis of complex traits, and appears to be effective for integration of genetic and genomic information.

Catechol-O-Methyltransferase (COMT) Polymorphism and response to Cognitive Remediation in Schizophrenia.

S. Kaushik[2], H. Lachman[4], J. P. Lindenmayer[1,2,3], S. Kaushik[1], A. Khan[1,5] 1) Psychopharmacology Research Un, Nathan Kline Institue, New York, NY; 2) Department of Psychiatry, Manhattan Psychiatric Center, New York, NY; 3) Department of Psychiatry, NYU School of Medince, New York, NY; 4) Department of Neuroscience, Albert Einstine College of Medicine, Bronx, NY; 5) Fordham University, Fordham, NY.

Background: Studies have indicated COMT val108/158met polymorphism associations with impairments in specific cognitive functions, in particular dorsolateral PFC dependent working memory and attention. Methods: 38 patients with schizophrenia were enrolled in a 12 week computerized neurocognitive rehabilitation treatment (CRT) program to evaluate the association of COMT genotype with the response to CRT. PANSS and Neurocognitive assessment using MATRICS Consensus Cognitive Battery were done at baseline and at end point. Analysis: 19 patients(3 males, 16 females) had Val/Val and 19 (1 male, 18 females) had Val/Met or Met/Met (Val+Met) genotype. Mean age for the whole cohort was 43.49 (SD=9.55) and mean length of illness was 18.38 months (SD=11.79). 58% were african americans, 24% caucasians, 35% hispanic and 2% asian. No significant differences were observed in demographic characteristics between Val/Val and Val+Met groups. RM ANOVA analysis showed significantly greater improvement in Global cognitive index (GCI)($p=0.05$) and Trail making test A (processing speed, $p=0.011$ and working memory, $p=0.049$) for the Val+Met group. Higher PANSS score were significantly correlated with Val/Val genotype ($p=0.044$). The correlation between effect sizes of improvement (higher GCI score and lower PANSS) was significant at $p=0.038$. Conclusion: The presence of Met allele was associated with significantly greater improvements in overall neurocognitive functioning after 12-weeks of CRT and indicates COMT polymorphism influences improvement of cognitive function after CRT. Due to the small sample size, the positive findings could be due to Type I Error. Therefore as we accrue a larger sample size we will be able to validate these findings and be able to determine if the two effects (i.e. improvement from CRT and COMT polymorphism) act at different levels.

Association analysis of seven tagSNPs in the endothelial nitric oxide synthase gene and acute mountain sickness.
P. Wang, MS. Koehle, JL. Rupert The University of British Columbia, Vancouver, BC, Canada.

BACKGROUND: Acute mountain sickness (AMS) frequently occurs in individuals who ascend to altitudes above 2500m. The recurrence rates of AMS in individuals suggest a genetic contribution to susceptibility to the condition. Nitric oxide (NO) is important in regulation of pulmonary vascular tone and may play a role in acclimatization to high altitude. Lower levels of exhaled NO have been demonstrated in individuals who adapt poorly to high altitude. There are a number of polymorphisms in the endothelial nitric oxide synthase (eNOS) gene which may affect NO levels. This study investigated the impact of variants in the eNOS gene on the susceptibility to AMS. METHODS AND STUDY DESIGN: 103 (80 male, 23 female) subjects were recruited at the 2005 Janai Purnima Festival at Lake Gosain Kunda at 4380 m in Lang Tang, Nepal. Seven tagSNPs in the eNOS gene (identified by HapMap) were investigated including the polymorphism Glu298Asp that previously has been associated with eNOS activity. AMS and non-AMS groups were determined both by Lake Louis Score (LLS) and by the more stringent criteria of clinical evaluation. DNA was prepared from buccal (cheek) cells, genotyped, and allele frequencies were compared between the AMS and non-AMS groups by Chi-Square analysis. RESULTS: The tagSNPs, rs1799983 (G/T; Glu298Asp)and rs1808593 (T/G), are associated with AMS when diagnosed by clinical evaluation. No association was found between AMS and alleles at other three tagSNPs (rs743507, A/G; rs3918188, C/A; rs7830, C/A. Genotyping SNPs rs1800781 and rs3918186 is ongoing. CONCLUSIONS: Our data suggest that the T (Asp) and G alleles at rs1799983 (Glu298Asp) and rs1808593 respectively may contribute to AMS susceptibility in the Nepalese.

Decreased expression of Hsp60 and Hsp70 by Caffeic acid phenethyl ester and induction of apoptosis in human oral cancer cells. *H. Tsai[1], T. Chen[2]* 1) Sch Medical Technology, Chung Shan Medical Univ, Taichung, Taiwan; 2) National Taichung Institute of Technology, Taichung, Taiwan.

Oral cancer is one of the ten most common cancers worldwide with a high morbidity and mortality. It is currently consider that dysregulated cell proliferation and apoptosis leads to the development of cancer. Heat shock proteins (Hsps) are overexpressed in various human cancers and involved in tumor cell proliferation and metastasis. To understand the role of Hsp in the pathogenesis of oral cancer, its expression was studied in oral squamous cell carcinoma. An oral cancer cell line, SCC4, was treated with caffeic acid phenethyl ester (CAPE) for 24 hours and then subjected to Hotech33258 staining, DNA fragmentation assay, RT-PCR and Western blot analysis for apoptosis. Results showed that SCC4 cells exhibited apoptotic features and fragmentation of DNA after a 24-hour treatment with CAPE. Furthermore, such CAPE-induced apoptosis was proved to be through an increased expression of proapoptotic protein Bax together with a decreased expression of antiapoptotic protein Bcl-2. In addition, CAPE reduced the heat shock response, leading to decreased levels of Hsp60 and Hsp70. Taken together, these data suggested that CAPE specifically suppresses Hsp60 and Hsp70 expression in oral SCC4 cells and blocks the inhibitory effects of this molecular chaperone on apoptotic cell death, indicating that Hsp60 and Hsp70 may participate in the CAPE-induced sensitization of oral cancer cells to anticancer drugs, which warrants further studies.

Systematic deep sequencing to identfy variants in GRM7 associated with age related hearing impairment. *A. F. Zebboudj[1], J. Ohmen[1], S. Bonneax[2], L. Van Laer[2], G. Van Camp[2], R. Friedman[1]* 1) House Ear Institute, Gonda Center for Cell and Molecular Biology, Los Angeles, CA, 90057; 2) Department of Medical Genetics, University of Antwerp, Antwerp, B-6210, Belgium.

Abstract: Studies employing whole genome genotyping are now routinely and reproducibly making positive associations between genetic variants (typically SNPS) and specific diseases and/or traits. Surrogate markers that "tag" individual haplotype blocks reduce the number of SNPs needed to capture genome-wide genetic variation from millions, to just a few hundred thousand. Haplotype blocks range in size from less than a kilobase to many hundreds of kilobases. While the identification of blocks associated with a given disease is important, the causal variant, and thus the true basis for the pathogenic condition, remains unknown. Identification of causal variants in these genome intervals will be an important next step for many of the studies currently in progress. We recently completed a genome-wide association study (GWAS) for genes associated with Age Related Hearing Impairment (ARHI). One of the most prominent genes identified in our analysis was GRM7. We fine mapped this association signal, and identified a significant stretch of genomic DNA sequence that appears to contain the ARHI association signal. To identify the mutation responsible for some part of ARHI pathogenesis, we have undertaken a strategy to sequence all the different haplotypes in this region to identify SNPs with minor allele frequencies as low as 1%. Once we have cataloged the different variants present in the individual haplotypes, we will genotype these variants in individual DNA samples, to accurately identify causal variants. Our strategy to sequence this region is to design overlapping 11kb amplicons, which can then be combined in equal molar amounts to provide complete coverage of this genomic region. We will then PCR amplify the entire genomic contig from each haplotype. Due to the enormous capacity of these next generation technologies, along with single molecule analysis, we can pool 50 to 100 DNAs representing each haplotype. The results of this strategy and effort will be presented.

Accurate discovery of expression quantitative trait loci under the presence of technical confounding factors. *C. Ye[1], H. Kang[2], E. Eskin[3,4]* 1) Bioinformatics Program, Univ California San Diego, San Diego, CA; 2) Department of Computer Science and Engineering, Univ California San Diego, San Diego, CA; 3) Department of Computer Science, Univ California Los Angeles, Los Angeles, CA; 4) Department of Human Genetics, Univ California Los Angeles, Los Angeles, CA.

Genome wide mapping of expression quantitative loci (eQTL) has successfully identified a large number of cis and trans associations. However, as several recent studies have demonstrated, technical confounding factors such as batch effects can complicate eQTL analysis by causing many spurious associations. Yet little is understood how these technical confounding factors affect eQTL analyses and how to correct for these factors. Through analysis of datasets with biological replicates we can motivate a statistical basis for the observation of spurious associations. We observe that pairs of expression arrays are often highly correlated due to technical confounding factors. Our analysis suggests that it is this inter-sample correlation structure inherent in expression data that leads to spurious associations. We propose a statistical method that corrects for the spurious associations caused by complex inter-sample correlation of expression measurements in eQTL mapping. Applying our Inter-sample Correlation Emended (ICE) eQTL mapping method to mouse, yeast, and human identifies many more cis associations while eliminating most of the spurious trans associations. The concordance of cis and trans associations has consistently increased between different replicates, tissues, and populations; demonstrating the higher accuracy of our method to identify real genetic effects.

Investigation of the colorectal cancer susceptibility region on chromosome 8q24.21 in a large German case-control sample. *J. Hampe[1], S. Buch[1], H. Völzke[7], W. von Schönfels[1], J. H. Egberts[2], B. Schniewind[2], M. Brosch[1], A. Ruether[4], A. Franke[4], M. Mathiak[5], B. Sipos[5], T. Henopp[5], S. Hellmig[1], M. Lerch[1], U. John[8], U. R. Fölsch[1], F. Fändrich[2], S. Schreiber[4,1], M. Krawczak[6], C. Schafmayer[2,3]* 1) General Internal Med, Univ Kiel, Kiel, Germany; 2) Departent of Surgery, UKSH Kiel, Germany; 3) POPGEN Biobank, UKSH Kiel, Germany; 4) Insitute of Clinical Molecular Biology, UKSH Kiel; 5) Insitute of Pathology, UKSH Kiel, Germany; 6) Institute of Medical Informatics and Statistics, UKSH Kiel, Germany; 7) Institute of Community Medicine, University Greifswald, Germany; 8) Deptartment of Medicine A, Universitiy Greifswald, Germany.

Background: Human chromosome 8q24.21 has been implicated as a susceptibility region for colorectal cancer (CRC) as a result of genome-wide association and candidate gene studies. Aims: To assess the impact of molecular variants at 8q24.21 upon the CRC risk of German individuals and to refine the disease-associated region. Patients and Methods: A total of 2713 patients with operated CRC (median age at diagnosis: 63 years) were compared to 2718 sex-matched control individuals (median age at inclusion: 65 years). Information on microsatellite instability in tumors was available for 901 patients. Results: Association analysis of SNPs rs10505477 and rs6983267 yielded allelic p-values of $1.42'10^{-7}$ and $2.57'10^{-7}$, respectively. For both polymorphisms, the odds ratio was estimated to be 1.50 (95% CI: 1.29-1.75) under a recessive disease model. The strongest candidate interval, outside of which significance dropped by more than four orders of magnitude, was delineated by SNPs rs10505477 and rs7014346 and comprised 17 kb. In a subgroup analysis, the disease association was found to be more pronounced in MSI-stable tumors (odds ratio: 1.71). Conclusion: Our study confirms the role of genetic variation at 8q24.21 as a risk factor for CRC and localizes the corresponding susceptibility gene to a 17 kb candidate region.

Identification of human and mouse alternative splicing of functional significance. *T. Imanishi[1], J. Takeda[1], Y. Suzuki[2], S. Sugano[2], T. Gojobori[1,3]* 1) BIRC, AIST, Tokyo, Japan; 2) Univ. Tokyo, Tokyo, Japan; 3) Natl. Inst. Genet., Mishima, Japan.

As a part of the project for developing an integrated database of human genes and transcripts, H-Invitational Database (H-InvDB; http://www.h-invitational.jp/), we analyzed comprehensive sets of human and mouse transcripts for alternative splicing variants of functional significance. As a result, we identified many human genes in which two or more alternative splicing variants are structurally conserved in mouse at the transcript level. This suggests that these alternative splicing variants are functionally important and contribute to the diversification of human and mouse proteome. On the other hand, there are more species-specific alternative splicing variants in human and mouse transcripts, implying that alternative splicing greatly serves evolutionary changes in gene structure. These results are made public in our database of human alternative splicing, H-DBAS (http://www.h-invitational.jp/h-dbas/).

Familial amyloid polyneuropathy (ATTR V30M): a monogenic disorder with an unusual large variability. *A. Sousa[1,2], A. Martins da Silva[3], L. Maia[3], T. Coelho[3]* 1) Dept Population Studies, ICBAS, Porto, Portugal; 2) UnIGENe, IBMC, Porto, Portugal; 3) Unidade Clínica de Paramiloidose, HGSA, Porto, Portugal.

Andrade first described FAP (1952) as a disease occurring between 25 and 35 yrs. He reported 64 patients, 13 of which had no family history of the disease. Later PE Becker established its AD mode of inheritance and interpreted isolated cases not as a de novo mutation but as the expression of incomplete penetrance of the gene in one of the parents. This hypothesis could only be tested after the finding of the mutation in 1985. Our aims were: 1) to estimate the number of probands with no affected parent at time of diagnosis; 2) to study age at onset of proband and its changes over time. Between 1939 and 2005, 2075 patients (525 families) were diagnosed at HGSA, all with V30M mutation. Families were classified as new when the proband reported no similar disease in earlier generations. 3) To assess the number of late-onset cases (o. 50y, thought to be so rare in earlier descriptions. Age-at-onset varied from 20-80 yrs (mean 37.1 in women, 32.4 in men). 209 probands (40%) had no affected parent at time of diagnosis. This type of family represented 68% of those diagnosed in last decade (only 27% before 1985). Mean age-at-onset of probands of new families was 46.0 yrs (vs. 32.3 yrs for classical families) and it has increased over time, being 49.5 yrs in last decade. Also, cases with late-onset are not rare: they represent 13% of all cases; also they aggregate in families and often descend from old asymptomatic carriers, what raises the hypothesis of genetic modifiers. Regarding FAP two realities coexist in Portugal: families with several generations of affected (where probands have classical onset) and probands with late-onset who report no similar disease in previous generations. The mutation may cross generations without clinical manifestations, then expresses as late-onset and later anticipation occurs, giving origin to families with classical onset of the disease.Since anticipation seems not to be due to TNR expansions, the question remains as how to explain anticipation in a disease due to a point mutation.

Dissection of optimal DNA dyes and amplicon length for high throughput mutation screening by real time PCR/high resolution melting analysis using the LightCycler480 System. *K. Naritomi[1,2], K. Yanagi[1], T. Kaname[1,2]*
1) Dept Medical Genetics, Univ Ryukyus Sch Medicine, Okinawa, Japan; 2) SORST, JST, Saitama, Janan.

We describe the optimal condition of DNA dyes for High-Resolution Melting (HRM) analysis using the LightCycler480 (LC480) System (Roche Diagnostics) with conventional Taq DNA polymerase. PCR was performed in presence of dyes, SYTO9, LCGreen Plus or EvaGreen. ***Background:*** LC480 System enable us to perform HRM analysis which could scan genetic variations such as single-nucleotide polymorphisms (SNPs) or mutations in PCR amplicons prior to sequencing by searching differences of thermal denaturation of a double strand DNA. As the optimal PCR condition for first step of genetic testing has been settled in most diagnostic laboratories, we tried to join the settled conditions to the new system. ***Methods:*** A 384-well plate was used to scan amplicons of the *Faciogenital dysplasia 1* (*FGD1*) gene. PCR was performed in the presence of double-strand DNA saturating dyes, SYTO9, LCGreen Plus and EvaGreen. Melting curves were analyzed with the LC480 Gene Scanning Software and reconfirmed sequences of DNA variants. ***Results:*** We show SYTO9 was most suitable for PCR-HRM analysis among them because of minimal affects on PCR amplification. The optimal concentration of SYTO9 was much less than previous reports. No clear relation was found in amplicon length and positions of mutations or SNPs in an Amplicon. Methods of DNA extraction dose not affect on HRM analysis. Amplicons included DNA dyes could be direct sequenced after purification using commercially available clean-up columns for PCR products. ***Conclusions:*** We concluded that SYTO9 is the most applicable for PCR-HRM analysis. No much effort was needed to adapt conventional PCR conditions to the new system.

A Genome wide association study of antioxidant vitamin levels identifies variants in the BCMO1 gene that alter vitamin A levels. *J. Perry[1], N. Rice[1], A. Matteini[2], M. N. Weedon[1], H. Lango[1], D. Hernandez[3], A. Singleton[3], A.-M. Corsi[4], J. Guralnik[3], S. Bandenelli[5], A. Cherubini[6], R. Semba[2], J. Walston[2], D. Melzer[1], L. Ferrucci[3], T. M. Frayling[1]*
1) Peninsula Medical Sch, Exeter, United Kingdom; 2) Johns Hopkins University, USA; 3) National Institute of Ageing, USA; 4) Tuscany Regional Health Agency, Italy; 5) Azienda Sanitaria di Firenze, Italy; 6) University of Perugia Medical School, Italy.

The role of oxidative stress and antioxidants in health, disease and ageing, is controversial. To improve the understanding of the control of antioxidant levels we performed a genome wide association study of 1200 individuals from the population based InCHIANTI study. We tested associations between 496,032 SNPs and eight anti-oxidant vitamin measures; alpha-tocopherol, gamma-tocopherol, lycopene, and alpha-carotene, beta-carotene, Zeaxanthin, lutein, and Beta-cryptoxanthin. We followed up SNPs reaching p<5x10-7 using 601 individuals from the WHAS study. We identified two associations that exceeded genome wide significance in a joint analysis. A cluster of SNPs, situated 5 of the BCMO gene, were associated with beta-carotene levels (p=1x10-10 in InCHIANTI, p=3x10-6 in WHAS, joint p=1x10-15) and other carotinoids (e.g. joint p=6x10-13 for lutein levels). The strongest association with vitamin E related measures was between a variant in the APOAV gene (S19W), known to alter triglyceride levels, and alpha-tocopherol levels (p=2x10-8 in InCHIANTI, p=0.006 in WHAS). This finding is consistent with the fact that vitamin E is a fat-soluble micronutrient carried by plasma lipoproteins, but shows that genetically determined lipid levels impact on vitamin levels. There were no associations between these variants and vitamin intake or secondary phenotypes including diseases analysed as part of the WTCCC. Our results provide the first evidence for common genetic variants that alter circulating levels of antioxidants. Understanding the genetic basis of vitamin levels should help clarify the role of antioxidants in health.

Genotyping Oculocutaneous Albinism -- a Case Report from an Ongoing Project. *D. Adams, M. Huizing, W. Gahl*
NHGRI, NIH, Bethesda, MD.

Objective: We are evaluating a cohort of patients with clinical evidence of oculocutaneous albinism (OCA), but no definitive molecular diagnosis. The cohort includes individuals who have unusual presentations, and who have been evaluated for other albinism-related disorders. We describe a pair of siblings for whom our ongoing project has yielded a new, definitive diagnosis. **Methods:** DNA sequence was obtained from PCR amplicons using custom primers. A known pseudogene complicates the sequencing of the TYR gene. TYR encodes the melanogenic enzyme tyrosinase. Allele-specific primers were used to differentiate TYR from the TYR pseudogene. **Results:** The two sisters were ages 2 y/o and 9 m/o at the time of presentation. The initial evaluation was conducted under a protocol for Hermansky-Pudlak Syndrome (HPS). HPS combines OCA with platelet dysfunction and the potential for pulmonary fibrosis and chronic colitis. In addition to OCA, both children had intermittent abdominal pain and easy bruising. Gastroenterological, pulmonary, hematological and ophthalmological evaluations were performed, greatly reducing the suspicion for HPS. Molecular analysis of the OCA2 (P) gene, associated with OCA, type 2 (OCA-2), revealed heterozygosity for p.S736L, a mutation previously described in OCA-2 patients. A tentative diagnosis of OCA-2 was assigned. Given the lack of a second OCA2 mutation, we added the siblings to our re-analysis project, revealing a homozygous p.G190fs mutation in the tyrosinase gene, associated with OCA, type 1. **Conclusion:** Technical progress has dramatically reduced the cost of DNA sequencing, allowing for more liberal use of sequencing in the search for definitive diagnoses. By sequencing all known OCA genes in our undiagnosed cohort, we hope to both assign additional diagnoses and discover candidates for new OCA-related genes. Clinical sequencing will allow rapid screening for common causes of OCA to confirm clinical diagnoses and to exclude more severe conditions. Future areas of research include characterization of pathogenicity for known variants and discovery of variants outside the coding sequence of OCA-related genes.

Evaluation of germline genomic profiling for colorectal cancer screening and risk prediction in data from the Assessment of Risk for Colorectal Tumors in Canada (ARCTIC) study. *J. Little, S. Hawken* Epidemiology and Community Medicine, University of Ottawa, Ottawa, Canada.

The genetic variants associated with colorectal cancer(CRC) in candidate gene and GWA studies mostly have low-penetrance. Collectively but not singly, multiple common,low-penetrance variants have potential utility in triage to alternative prevention strategies. For example, genetic information could be used in targeting individuals at elevated genetic risk for enhanced screening surveillance. Using a candidate gene approach, we identified a panel of common, polymorphic germline variants associated with CRC with some consistency, based on original research articles, meta-analyses and systematic reviews. This list of variants was then used to develop and test risk prediction models in a population-based case control study with genotyping of over 600,000 SNPs in 1200 CRC cases, and 1200 controls (the ARCTIC study). Approximately 80 variants were identified for evaluation in ARCTIC, but only about 1/3 were adequately captured in the data due to: low minor allele frequency, lack of coverage in the SNP panels, and the fact that a number of variants were not simple SNPs (e.g. microsatellites, deletions/ insertions). Model building proceeded in the ~20-30 SNPs available. Univariate odds ratios (OR) for homozygotes for uncommon variants versus homozygotes for common variants ranged from 0.67-2.16, and ORs for heterozygotes versus homozygotes for common variants ranged from 0.67-1.22. Minor allele frequency ranged from 1-50%. Although the genotype call rate was high for individual SNPs, missing data became an important issue in multivariable models, and hence we employed data-imputation techniques. Multivariate logistic regression analysis was used, with cross-validation techniques employed to address overfittingan important issue given our goal of developing generally applicable risk prediction models. Results showing promising predictive utility in the reduced set of available SNPs will be discussed. Next steps involve genotyping the variants not available in ARCTIC, and accessing additional data sources through collaborators, both for pooled analyses and for risk model validation.

Identification of biological targets for a microRNA molecule, miR-145 in prostate cancer cell lines. *M. Ozen[1,2], A. Uzumcu[1], O. F. Bayrak[1]* 1) Dept of Medical Genetics, Yeditepe University Medical School and Yeditepe University Hospital System, Istanbul, Turkey; 2) Dept Pathology, Baylor College of Medicine, Houston, TX.

MicroRNAs (miRNAs) are small (20-22 nucleotide) regulatory RNAs which might regulate gene expression transcriptionally or translationally. There is emerging evidence that miRNAs are involved in cancer pathogenesis. A number of studies have detected frequent alterations of miRNA expression in a variety of human malignancies including prostate cancer. We have recently demonstrated a widespread deregulation of miRNA expression in human prostate cancer. In this study, we have analyzed the status of one of the selected miRNAs, miR-145 in human prostate cancer cell lines DU-145, LNCaP and PC3. We have transfected these cell lines with precursor miR-145 and anti miR-145 molecules and compared the gene expression profiles by using Agilent whole genome expression arrays. Data analysis is carried out by the help of Agilent Gene Spring GX software package. We have also compared the targets for miR-145 identified biologically in prostate cancer cells and in three different databases. Experiments on verification of select targets and the biological effects of these targets on prostate cancer cell lines are in progress. The results obtained in these studies might identify new markers that can be used for prostate cancer progression and as new targets for prostate cancer therapy.

Significant association of MECP2 alleles with autism. *R. D. Delahanty, E. Crawford, D. G. Hwang, L. Jiang, K. Luci, J. S. Sutcliffe* Ctr for Human Genetics Res, Vanderbilt Univ, Nashville, TN.

Mutations in the methyl CpG binding protein 2 (MECP2) gene cause Rett syndrome (RTT; OMIM 312750), accounting for up to 95% of cases. Animal studies have demonstrated that MeCP2 is essential for normal brain development. While RTT has a distinct, known etiology, it has nevertheless been categorized as a Pervasive Developmental Disorder, as has autism and its spectrum diagnoses; this reflects the shared phenotypic features of these two disorders, and has fostered speculation that they may also share biological connections as well. Autism, like RTT, is a neurodevelopmental disorder. Narrowly defined autism affects ~1 in 500 individuals, while the broader autism spectrum disorders (ASDs) affect ~1 in 150 individuals. Key clinical features of autism include deficits in reciprocal social interaction, communication and patterns of repetitive behaviors and restricted interests. Autism affects males more commonly than females at a ratio of 4:1. In contrast, the vast majority of RTT cases are female, owing to lethality in males fully deficient for MECP2. To ask whether common alleles of MECP2 might contribute to autism, we conducted an association study using SNP markers to index common haplotypes across the MECP2 locus. These SNPs were genotyped in a group of 962 combined simplex and multiplex families ascertained for idiopathic autism. FBAT and x-APL application were used to conduct a family-based test to evaluate our hypothesis. We found that multiple SNPs in a block of linkage disequilibrium showed strong association using both narrow (e.g. P=0.0007) and broad (e.g. P=0.0054) diagnostic criteria. These associations remain significant even after a conservative Bonferroni correction. A more in depth, albeit preliminary analysis of these data indicate an ancestry effect, with the association driven by a non-European subset of families. Resequencing of MECP2 exons in a set of 192 unrelated cases and 192 controls does not indicate a burden of rare mutations in the autism sample. To our knowledge, this is the first report suggesting that common alleles in MECP2 may confer susceptibility to autism.

Generation of a Human miRNA Tissue Atlas. *B. B. Gardiner[1], G. Kolle[1], N. Cloonan[1], J. Gu[2], E. Nourbakhsh[1], K. Lea[2], S. Heater[2], S. Kuersten[2], S. M. Grimmond[1]* 1) Institute for Molecular Bioscience, University of Queensland, Brisbane, Queensland, Australia; 2) Ambion, Inc. - An Applied Biosystems Business, Austin, TX 78744.

The involvement of miRNAs in many cellular and developmental processes is well established and the elucidation of their roles depends on a complete catalog of miRNAs and a more thorough understanding of their expression patterns. To address these issues a robust method for expression profiling and novel miRNA discovery is required. We have previously used a shot-gun sequencing approach in conjunction with the Applied Biosystems SOLiD technology to assess gene activity and transcriptional complexity (Cloonan et al., 2008). In contrast to array based approaches direct sequencing of RNA populations does not suffer from the issues of cross-hybridization, and can be utilized for the discovery of novel sequences. Further, short read sequence approaches are especially suited to the discovery and analysis of miRNA sequences. We have generated a sequence based expression atlas by surveying the miRNA fraction across nine human tissue samples (heart, brain, liver, testes, spleen, kidney, thymus, lung and ovary). Individual barcoded libraries were prepared using an RNA ligation based approach and pooled for SOLiD sequencing. Our sequence data reveals that tissue restricted miRNA expression can be detected and quantified by this approach, which also allows for the identification of variations in the mature miRNAs as well as the identification of novel miRNAs.
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Development of a national certification program for hemoglobinopathy service counselors Joseph Telfair*, Sonya Ross **, Nancy Callanan*, Eileen Miller,* UNCG* , SCDAA.** *J. Telfair* Public Health, University of North Carolina at Greensboro, Greensboro, NC.

In Fall 2004, the Sickle Cell Disease Association of America (SCDAA) engaged Professional Examination Service (PES) to perform consulting services with regard to the development of a national certification program for hemoglobinopathy service counselors. An overall description of a hemoglobinopathy service counselor, and a description of the domains and tasks performed by such counselors as well as the knowledge required was crafted and subsequently validated via electronic survey disseminated to subject-matter experts nationwide. A review of the quantitative results from the survey indicates that the domains of practice are important areas of practice that comprise the work time of hemoglobinopathy counselors. Moreover, the topic areas comprise knowledge and skills that are required in practice and that make a very important contribution to protecting the client/public from health-related harm. Accordingly, The domains and tasks identified in connection with the profile of the hemoglobinopathy service counselor provide a valid and comprehensive description of the work of such practitioners. The knowledge base identified in connection with the topic areas can be used as the basis of both curriculum development and review initiatives and test development initiatives. The skill based identified in connection with the topic areas can be used as the basis for evaluating the competencies of hemoglobinopathy service counselors during training and as a pre-condition for certification. The validated description of practice, including domains, task, topics, and knowledge and skills provides a reasonable basis for related accreditation and certification initiatives, should the SCDAA elect to move forward in either direction.

Sepsis in premature infants: investigation of candidate genes. *M. E. Cooper[1], A. S. Abu-Maziad[2], K. L. Schaa[2], M. L. Marazita[1], J. M. Dagle[2], J. C. Murray[2]* 1) University of Pittsburgh, Pittsburgh, PA; 2) University of Iowa, Iowa City, IA.

Sepsis is a serious bacterial infection that is a significant cause of morbidity and mortality in neonates. In this study we examined 48 SNPs in 16 candidate genes on 12 autosomal chromosomes. The sample is 644 nuclear families, with 82 premature infants diagnosed with sepsis, 261 with suspected sepsis and 216 with no sepsis. Birth weight, gestational age, delivery type, race/ethnicity, number of days on oxygen/ventilator, rupture of membranes details, bacterial gram positive/negative episodic details and chorioamnionitis status were collected. **Methods**: Allelic, genotypic and haplotypic transmission disequilibrium tests (TDT) were performed. Within the set of premature infants, various tests (Chi Square, ANOVA, correlations, regression) were used to assess association between covariates and sepsis defined as definite sepsis with/without suspected sepsis. **Results**: All biological measures are highly correlated (all p0.0001) and vary significantly between the 3 sepsis groups (ANOVA p0.0001). A model with gestational age and gender as main effects is the best predictor of definite sepsis diagnosis. TDT analysis reveals that definite sepsis is associated with PLA2G2Ars1891320s common C allele (p=0.04) and C/C genotype (p=0.08) but that suspected sepsis is associated with the rare T allele and the C/C genotype is protective (p=0.02). Definite sepsis is associated with TLR5rs5744105s C allele (p=0.05) and the G/G genotype is protective (p=0.07); the no sepsis group is associated with the G allele (p=0.02) and the C/G genotype (p=0.04) but the C/C genotype is protective (p=0.004). Two SNPs in the IL10 and TLR2 genes are associated with definite sepsis (p=0.05) and 3 other SNPs are associated with suspected sepsis group but not in the other groups. Conclusions: Although the p-values are only minimally significant even without multiple test adjustment, the results are intriguing. It appears that the C allele in SNPs from the PLA2G2A and TLR5 genes are relevant to sepsis and the opposite allele are associated with non-sepsis. Further studies are needed to explore the role of these genes in the occurrence of sepsis in premature neonates. Grant:#HD52953.

Rapid Screening of genetic factors associated with warfarin dosage using a nanofluidic system. *R. Ramakrishnan[1], A. Mir[1], H. Sagreiya[2], R. D. Altman[3]* 1) R&D, Fluidigm Corp, S San Francisco, CA; 2) Stanford Medical School, Stanford CA; 3) Department of Bioengineering, Stanford University, Stanford, CA.

There is a need for systems allowing medium multiplexing platforms with high throughput, excellent call rates, high concordance and low cost. In the current study we demonstrate the use of one such system, utilizing unique nanofluidic products in a clinical research study that analyzed the genetic and clinical determinants of warfarin dose. We demonstrate the use of Integrated Fluidic Circuits (IFCs), called dynamic arrays, in a study to genotype unique human DNA samples on 28 different SNP assays, covering the VKORC, CYP2C9 and the CYP4F2 genes, using nanoliter volumes of reagents. The DNA samples screened included DNA case control samples extracted from saliva of patients currently undergoing warfarin therapy, at the Stanford Oral Anticoagulation Clinic (OAC) and the Stanford Hematology Clinic. Each dynamic array IFC systematically combines samples and assays into 9216 reactions in a single chip run. Each chip was thermocycled, imaged and analyzed using a BioMarkTm system. Call rates of greater than 99.5% and high concordance values were achieved. Calls were validated by selected genotyping of the same samples on the ABI 7900. We have also determined copy number variation changes associated with these patients on the BioMark system using a second different IFC, known as the digital array. Each digital array is capable of reliably discriminating between copy numbers which are only 15% different. The excellent call rates and high concordance, combined with the combination of samples and reagents in nanofluidic chips, provides a formidable screening tool. It is possible that this research could lead to improved warfarin dosing policies at Stanford Hospital and Clinics in the future.

Dysfunctional MeCP2 causes microtubule defects. *S. Alka[1], R. Scaife[1], J. Christodoulou[2], P. Zhang[1], G. Pelka[3], P. Tam[3], H. Leonard[4], G. Matthijs[5], M. Kavallaris[6], D. Ravine[1,7]* 1) Western Australian Institute for Medical Research, Perth , Western Australia, Australia; 2) Western Sydney Genetics Program, Childrens Hospital at Westmead, Sydney, NSW, Australia; 3) Children's Medical Research Institute, 214 Hawkesbury Rd, Westmead NSW 2145 Australia; 4) Telethon Institute for Child Health Research, Centre for Child Health Research, University of Western Australia, 100 Roberts Road, Subiaco, Perth, WA 6008, Australia; 5) Laboratory for Molecular Diagnostics, Center for Human Genetics, University of Leuven, Herestraat 49, B-3000 Leuven, Belgium; 6) Children's Cancer Institute Australia for Medical Research, High Street, Randwick, Sydney, NSW 2031, Australia; 7) School of Medicine and Pharmacology, University of Western Australia, Level 5/6, MRF building, Rear 50 Murray Street, Perth WA 6000, Australia.

Methyl CpG binding protein 2 (MeCP2) is known to mediate gene expression by several mechanisms within the nucleus. MeCP2 also has a recently recognised cytoplasmic distribution, which is of unknown functional significance. Here, we report that MeCP2 maintains microtubule stability via the tubulin de-acetylation pathway. We show that MeCP2 associates with microtubules in the cytoplasm as well as on the mitotic spindle and within the midbody remnant. We have found that microtubule inhibitors alter the cytoplasmic distribution of MeCP2 in wild type cells. In a MECP2 mutant cell line, we have detected impaired microtubule stability and dynamics together with reduced amounts of acetylated tubulin. siRNA knockdown of MeCP2 in wild type cells is associated with reduced amounts of acetylated tubulin, whereas MeCP2 overexpression heralds a rise in the level of acetylated tubulin. We have since identified an interaction between MeCP2 and the microtubule deacetylase histone deacetylase 6. We propose that that MeCP2 maintains microtubule stability by inhibiting the tubulin deacetylase activity of HDAC6.

Skewed X-inactivation in Female Carriers of Menkes Disease Associated with Neuropsychiatric Abnormalities.

V. Desai[1], A. Donsante[1], M. Martensen[2], K. Swoboda[2], S. G. Kaler[1] 1) Unit on Pediatric Genetics, NICHD, NIH, Bethesda, MD; 2) Pediatric Motor Disorders Research Program, University of Utah School of Medicine, Salt Lake City, UT.

Menkes disease is an X-linked recessive neurogenetic disorder caused by mutations in a gene, ATP7A, located at Xq13, that encodes a copper-transporting P-type ATPase. Deficiency of the gene product causes abnormal cellular copper transport and decreased activities of copper-dependent enzymes. ATP7A plays a significant role in the development and maintenance of the central nervous system (CNS), as evidenced by the marked neurodevelopmental abnormalities in affected patients. Female carriers of this condition are typically healthy and do not manifest clinical or biochemical phenotypes. We studied a large Utah family in which an ATP7A mutation (del exon 13-14) segregates. We identified a 5,557 base deletion in the proband and mapped the breakpoint using long polymerase chain reaction (long-PCR) and stepwise sequencing. We developed an assay to amplify a 684 bp junction fragment in the proband, his mother, and maternal grandmother (obligate carriers). We then applied this assay to evaluate six other at-risk females in the family, as well as a newborn male. In addition to the two obligate carriers, we detected the junction fragment in four of the other six at-risk females. The newborn male, born to one of the identified carriers, did not show the fragment, ruling out Menkes disease. X-inactivation studies showed moderately skewed (n=2) to highly skewed (n=4, 100:0 in 3) patterns among the six carriers, and random X-inactivation in two non-carriers. Serial neurocognitive evaluations during childhood and early adulthood revealed significant abnormalities in several females with del exon 13-14. Our results provide evidence that heterozygosity for a severe ATP7A mutation, in conjunction with nonrandom X-inactivation, may be associated with significant clinical abnormalities in females. We speculate that the 5.5 kb deletion in ATP7A interferes with cis-limited silencing by Xist RNA, or is genetically linked with a second mutation at Xq13, viz., in the Xic locus, accounting for the marked skewing observed.

Community Centered Family Health History: A model and a resource. *J. O'Leary, V. Edelson, S. Terry* Genetic Alliance, Washington, DC.

Significant stigma exists in communities and families surrounding health information. Privacy concerns and fears of discrimination can make it difficult to discuss health within the family, let alone with a healthcare provider. Yet the family plays an important role in shaping a person's habits and encouraging healthy behaviors, and a provider can recommend lifestyle modifications based on family risk. Community Centered Family Health History models how adaptation of resources and a broad definition of community can produce infinite individualized access points to health information. The Does It Run In the Family? health history toolkit addresses common, complex conditions alongside single gene disorders to foster the understanding that all people are affected by their genetics, in ways that might be surprising. Communities in the project customized family health history materials and translated community experiences into a customizable online version of the Does It Run In the Family? toolkit. The online tool allows users to input personal stories, photographs, quotes, resources, and health condition descriptions and statistics - or choose from libraries of such information - to easily create community-specific resources.

Technical variation and whole genome data quality: A systematic approach to "cleaning" genotype data for analysis. *R. Lazarus, B. Raby, E. K. Silverman, B. Klanderman, C. Hersh, S. T. Weiss* Channing Laboratory, Brigham and Women's Hospital; Center for Genomic Medicine, Brigham and Womens Hospital; Harvard Medical School, Boston, MA.

Genotype measurement error is a potential source of bias in genetic association studies. From sample collection to assay, every step in high throughput genotyping is highly optimized, so even minor departures from optimal conditions are likely to decrease genotype quality, and some kind of data cleaning is nearly always performed before analysis. Subjects and markers lying at the extremes of the distributions of technical measures, including missingness rates, departures from Hardy-Weinberg equilibrium (HWE), Mendelian error rates and duplicate genotyping discordance rates, are candidates for removal as part of a quality control process, because these characteristics suggest non-optimal technical conditions associated with process control variation and increased genotype error rates. These candidates can easily be identified with *in-silico* methods, but guidance on optimal strategies for data cleaning is lacking. We evaluated and compared measures of technical quality variation in whole genome data from 1200 subjects in the CAMP study, and explored their potential contribution to systematic data cleaning strategies, using departure from the expected uniform distribution of p-values under the null, as a figure of merit for a range of thresholds for each measure. In a principal components analysis (PCA) of markers, ¾ of the total variance was explained by the first 2 components. The proportion of Mendelian errors, the missing fraction and discordance rates for duplicate measurements loaded heavily onto the first component, the logarithm of the Hardy-Weinberg p-value in founders loaded equally onto both, while the minor allele frequency (MAF) loaded mostly onto the second component. MAF affects power to detect departures from HWE but appears not to affect the other commonly used measures of technical variation. We conclude that PCA may be used to identify outlier markers in technical variation PCA space for removal during quality control before analysis. Support: HG003646, HL065899, HL083069.

Genomic profiling of high grade primary brain tumor, the Gliomas: A cytogenomic study by FISH. *P. Singh-Kahlon, L. Montella* Cytogenetics, Genzyme Genetics, 521 W. 57th St, #5, New York, NY 10019.

Diffuse gliomas are the primary brain tumor, graded as per WHO classification (in high grade category), as oligodendrogliomas, asrocytomas, anaplastic oligodendrogliomas & mixed oligoastrocytomas. Genomic profiling of these tumors has prognostic significance. It has been shown that loss of heterozygosity (LOH) by the codeletion of two gene regions, EGLF and TP73 located at 1p36 and GLTSCR1, GLTSCR2, CRX on 19q13, results in improved prognosis for the above mentioned high grade diffuse gliomas. FISH based cytogenomic studies with 1p36 and 19q13 probe sets were conducted on over 150 samples of paraffin block specimens. Standard FISH hybridization methodology (Vysis) used for block tissue samples and modified in our Genzyme laboratory was employed. FISH data analysis showed nearly 76% of the total samples as abnormal. Amongst the abnormals, 55.3% had loss of heterozygosity (LOH) for both 1p and 19q i.e concurrent codeletion, 5.3% showed deletion of 1p alone, and 1% deletion of 19q only. Multisomy of 1p and 19q was present in 24.4% cases, while 13.8% showed complex abnormal FISH patterns. Correlation of the observed genomic aberrations with clinical subtypes and prognostic implications for high grade gliomas shall be elucidated.

PCR/high-resolution melting analysis for rapid and sensitive gene scanning of the faciogenital dysplasia gene, FGD1. *T. Kaname[1,2], K. Yanagi[1], Y. Chinen[3], K. Naritomi[1,2]* 1) Dept Medical Genetics, Univ Ryukyus, Nishihara, Japan; 2) SORST, JST, Kawaguchi, Japan; 3) Dept Pediatrics, Univ Ryukyus, Nishihara, Japan.

It is important to establish an easy and reliable system to detect mutations or variations for genetic testing. High-resolution melting analysis (HRM analysis) is a method, which allows simple and rapid detection of gene variations. We constructed a sensitive system for detecting gene variations in *FGD1*. The *FGD1* gene is a responsible gene for Aarskog-Scott syndrome (AAS), which is an X-linked disorder characterized by short stature, dysmorphic facial appearance, brachydactyly, shawl scrotum, and sometimes neurobehavioral impairment. We set up PCR/HRM system for all the exons of *FGD1* using LightCycler 480 Instrument (Roche). Then we evaluated the PCR/HRM in the screening seven mutations of *FGD1*, which we found in AAS patients previously, plus variations of *FGD1* in five sporadic patients, two families, and 48 controls. The PCR/HRM discriminated all the *FGD1* mutations studied from wild-type DNA. In control individuals, four polymorphisms and three unknown variations were found in the *FGD1* gene. Besides, the PCR/HRM discriminated not only four haplotypes in exon 14, but also between heterozygous and hemizygous or homozygous of those haplotypes. The system is a valuable method for rapid and reliable scanning of *FGD1* gene variations and is applicable to high-throughput genetic testing.

A novel cassette exon flanked by schizophrenia-associated SNPs in the dopamine transporter gene. *M.Y-M. Chen[1], K. McCann[1], P. Papasaikas[1], M. E. Talkowski[3,4], M. Bamne[3], L. McClain[3], D. Lewis[3], V. L. Nimgaonkar[3,4], A. J. Lopez[1,2]* 1) Dept Biological Sciences, Carnegie Mellon University, Pittsburgh, PA; 2) Lane Center for Computational Biology, Carnegie Mellon University; 3) Dept Psychiatry, Univ Pittsburgh, Pittsburgh, PA; 4) Dept Human Genetics, Univ Pittsburgh, Pittsburgh, PA.

The dopamine transporter (*SLC6A3*, DAT) is a critical gene involved in the re-uptake of dopamine from the synapse. Previous studies identified statistical associations between common intronic sequence polymorphisms in *SLC6A3* and schizophrenia in two large Caucasian samples (see Talkowski et al. abstract and Talkowski et al 2008). This prompted us to examine the *SLC6A3* sequence for possible alternative exons or recursive splicing elements that might have been missed by previous molecular analyses. Using a primate-specific computational model, we predicted a novel 108 nt cassette exon that is defined by a potential recursive splice site and a standard 5 splice site motif within intron 3. This exon was flanked within 600 nucleotides by four schizophrenia-associated SNPs, three of which fell within computationally predicted splicing regulatory signals. We verified alternative splicing of the cassette exon (E3b) in cell transfection experiments in both its normal intron context and in a heterologous splicing reporter system. Results of mutagenesis tests are consistent with a recursive splicing mechanism for E3b. We also verified alternative splicing of exon E3b in endogenous *SLC6A3* transcripts from adult human brain and lymphocytes using RT-PCR assays. Because E3b introduces multiple in-frame stop codons into the mRNA, it truncates the DAT protein and may serve a negative regulatory function. This might also explain why inclusion of E3b had not been detected previously, since it might trigger nonsense-mediated decay of the corresponding mRNA. A potential exon E3b is conserved among primates and many other placental mammals but it appears to have been lost in the Glires. Experiments are under way to test whether the frequency of inclusion of exon E3b is altered by alleles associated with schizophrenia risk at the flanking SNP positions.

PhenX: Consensus Measures to Facilitate Cross-Study Analysis for Genome-wide Association Studies. *C. M. Hamilton[1], E. Ramos[2], R. Kwok[1], D. Maiese[1], D. Wagener[1], W. R. Harlan[3], J. Haines[4]* 1) RTI International, Research Triangle Park, NC; 2) National Human Genome Research Institute, Bethesda, MD; 3) National Library of Medicine, Chevy Chase, MD; 4) Center for Human Genetics Research, Vanderbilt University, Nashville, TN.

Genome-wide association studies (GWAS) measure hundreds of thousands of single nucleotide polymorphisms (SNPs) across the genome and relate them to common diseases and traits. Once an individuals genome has been comprehensively characterized, it can potentially be related to any trait, not just the trait that was initially investigated. Despite the vast potential of GWAS, opportunities for cross-study comparisons have been severely restricted by the lack of standardized phenotypic and environmental measures, even though many risk factors (e.g., obesity, smoking, low socioeconomic status) are common to multiple diseases. PhenX (consensus measures for Phenotypes and eXposures) was initiated to enhance cross-study analyses of GWAS and other large-scale genomic research efforts. The PhenX Steering Committee has identified 20 high priority research domains. Over the course of the three year project, experts from across the scientific community will participate as members of Working Groups (WGs) to address these research domains. Each WG will propose a set of high-priority measures that will be vetted with the larger research community through web-based surveys. Ultimately, the PhenX Toolkit will make the selected measures available to the scientific community. The PhenX Toolkit will make it easy for researchers to incorporate the recommended measures in proposed or ongoing genome-wide studies. To date, five PhenX WGs are in progress: Demographics, Anthropometrics, Alcohol, Tobacco and Other Substances, Cardiovascular, and Diet and Nutrition. The input of the scientific community is needed to ensure that the measures included in the PhenX Toolkit will be widely accepted and readily incorporated into ongoing and future research efforts. Investigators are encouraged to find out more details about PhenX and to participate in the web-based surveys at www.PhenX.org. Supported by: NHGRI, Award No. 1U01 HG004597-01.

Etiology of 200 infertility cases. *C. Trujillo[1,2], S. Ashour[1,3], S. B. El Badawi[1]* 1) Dept Genetics Erfan & Bagedo Hospital, Jeddah, Saudi Arabia; 2) Dept Genetics, CES Inst, Medellin, Colombia; 3) Andrology Department, Cairo University, Egypt.

Objectives: According to the WHO 15% of all couples attempting to have children are infertile. The male factor alone explains 30% and a combination of male and female factors is 20%. Genetic factors clearly contribute a big portion of the infertility etiology. Methods:Clinical evaluation included, detailed medical history and physical examination.Testicular volume was measured by orchidometer.Semen analysis was performed according to the WHO guidelines.The diagnosis of azoospermia was established after centrifugation.LH,FSH and Testosterone were measured by immunometric assays with commercial kits.Chromosomal analysis was performed using G banding;FISH studies was used in all cases of mosaicism and in 46,XX males, using centromeric probes for the X and Y chromosomes and SRY probe.Molecular testing for Y chromosome microdeletion of the AZF regions were evaluated by PCR using the European Academy of Andrology (EAA) and (EMQN)laboratory guidelines. Results:Total of 200 patients were studied, 121 were oligospermic, 79 Azoospermic.Chromosome abnormalities found in 30 azoospermic and in 7 oligospermic; including 47,XXY 20 cases, 4 mosaic cases (47,XXY/46,XY/45X, 47,XXY/46,XY and 46,XY/45,XY).Two patients had a karyotype 46,XX and The SRY was detected by FISH.One patient had a karyotype 46,XY with absence of the SRY gene.Y microdeletion were found in 4 cases of azoospermia, two in the AZFc, and one in both the AZFb-c region. In two cases of oligospermia AZFc mutation were found. Three patients with obstructive azoospermia one was heterozygous for the 508 mutation, and one was compound heterozygous compound heterozygous. Other causes of infertility in our patients were Werner Syndrome (one patient), del 19ptet. Conclusion:The overall rate of genetic abnormalities found in this study was 30%.Infertile men with a normal karyotype have an increased risk of chromosomally abnormal sperm and children.New techniques may help to identify this cases.Whether this further will help our patients to be included in assisted reproductive programs will be worth to consider in future studies.

PhenX: Demographic Measures for Cross-study Analyses. *M. Cockburn[1], V. Bonham[2], O. Carter-Pokras[3], G. Gee[4], R. Kington[5], N. Lange[6], D. Makuc[7], P. Kraft[8], M. Phillips[9], R. Kwok[9], D. Maiese[9], D. Wagener[9], E. Ramos[2], C. M. Hamilton[9]* 1) Department of Preventive Medicine, USC, Los Angeles, CA; 2) NHGRI, Bethesda, MD; 3) University of Maryland, College Park, MD; 4) UCLA School of Public Health, Los Angeles, CA; 5) NIH, Bethesda, MD; 6) Schools of Medicine and Public Health, Harvard University, Belmont, MA; 7) National Center for Health Statistics, CDC, Hyattsville, MD; 8) Harvard School of Public Health, Boston, MA; 9) RTI International, RTP, NC.

The potential for cross-study comparisons in genome-wide association studies (GWAS) is severely restricted by the lack of standardized or comparable phenotypic and environmental measures. PhenX (consensus measures for Phenotypes and eXposures) is a consensus-building effort to choose high-priority phenotypic and environmental exposure measures which are of public health significance and suitable for GWAS. The goal of PhenX is to improve the ability of research groups to combine their data, thus increasing statistical power and the ability to detect genes associated with common complex diseases. The selected high-priority measures will be made available to the scientific community via the PhenX Toolkit. The PhenX Steering Committee selected Demographics as the first domain to be addressed by a Working Group (WG). The PhenX Demographics WG is composed of researchers whose expertise represents the wide breadth of the field. The selected demographics domain measures assess fifteen elements including: age; race; ethnicity; gender and ancestry. The Demographics WG recommended a set of measures and associated measurement protocols. Opinions on the proposed measures were sought from the research community through a web-based survey, the results of which were then used to define a small set of high priority measures. Measures were chosen on the basis of several criteria including: clearly defined; well established; broadly applicable; and a low burden to participants and investigators. The PhenX Steering Committee has identified 19 additional research domains to be addressed by PhenX Working Groups over the course of the three-year project. Supported by: NHGRI, Award No. 1U01 HG004597-01.

Bipolar disorder as presenting clinical feature associated with the 3243 AG mutation in the mitochondrial tRNA Leu(UUR) gene. *F. Scaglia[1], C. W. Brown[1], R. Diaz Millan[2], L. J. Wong[1]* 1) Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX; 2) University of Guatemala School of Medicine, Guatemala City, Guatemala.

Mitochondrial cytopathies are a genetically, biochemically, and clinically heterogeneous group of disorders associated with abnormalities of oxidative phosphorylation that affect organs and tissues highly dependent on energy metabolism such as the central nervous system, the skeletal muscle and the heart. These disorders demonstrate a wide range of central nervous system involvement and may be accompanied by focal brain necrosis, dementia or static encephalopathy; however, it is increasingly recognized that they may be associated with psychiatric illness and in particular affective disorders. The 3243 AG mutation in the mitochondrial tRNALeu(UUR) gene has been associated with several clinical phenotypes including mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS), Leigh syndrome, progressive external ophthalmoplegia, and diabetes and deafness. Here we report a family carrying the common 3243 AG mutation and whose main presenting clinical feature was unusual behavior later characterized as bipolar disorder. The proband first presented at age 16 years with unusual behavior that was diagnosed as bipolar disorder. Subsequently, he developed multiorgan dysfunction that included seizure disorder, sensorineural hearing loss, short stature, and dilated cardiomyopathy and was found to harbor the 3243 AG mutation. His family history was relevant for bipolar disorder as the presenting clinical feature on his mother, maternal aunt and maternal grandmother who were also found to harbor this mutation. The atypical clinical presentation in this family illustrates that psychiatric illness may be associated with mitochondrial dysfunction expanding the clinical spectrum of mitochondrial cytopathies.

Estimate of the local FDR in genome-wide association studies. *J. Bukszar, E. J. van den Oord* Virginia Commonwealth University/ Medical College of Virginia Campus 410 North 12th Street Richmond, VA. 23298.

We present a method that estimates the individual effect sizes (IES) in genome-wide association studies. A novel and key element is that we use the real likelihood function of the multiple tests rather the one based on the frequently used mixture model. Interestingly enough, this latter one would result in fairly inaccurate estimate even for arbitrarily high sample size. Our IES estimates enable us to estimate the posterior probability that a marker has no effect, i.e. the local FDR, accurately. Further applications of IES, such as the estimate of Storeys q-values, will also be discussed.

Newborn screening in Fragile X syndrome. *F. Tassone[1,2], P. J. Hagerman[1,2], R. J. Hagerman[2,3]* 1) Department of Biochemistry and Molecular Medicine, University of California, School of Medicine, Davis, California, USA; 2) M.I.N.D. Institute, University of California Davis Medical Center, Sacramento, California, USA; 3) Department of Pediatrics, University of California, School of Medicine, Davis, California, USA.

Screening for the FMR1 mutations has been a topic of considerable discussion since the FMR1 gene was identified. However, Fragile X has not been recommended for newborn screening mainly because of the lack of an accurate screening test and of data on potential benefits. We have recently developed an improved PCR method for the identification of premutation and full mutation alleles for the FMR1 gene. The method is an inexpensive, accurate, and quick and can be performed on a number of sample templates including, and more importantly, blood spots. We have applied this method for international screening. Specifically, we have screened 5267 anonymous blood spot samples from newborn males from the center-northwest region of Spain. One important outcome from this study is that the frequency of premutation alleles (1 per 250) appears to be higher than previously reported. This is of importance, especially in view of the different phenotypic involvement observed in carriers of premutation alleles, including neurological problems such as FXTAS. Here, we present data on the frequency of premutation/full alleles found in this population and their size distribution. We have also used this technology to a pilot high risk screening program of individuals with autism and/ or intellectual disabilities and family members of a proband with fragile X initiated in Guatemala. This project is a prototype for future screening endeavors. Results from our pilot program in both Spain and Guatemala will lend strong support for implementing this technology for rapid screening to a much larger scale population screening.

L1c2a, the (African) Haplogroup With The Longest Mitochondrial Genome! *K. Ritchie[1], U. A. Perego[1,2], A. Achilli[2,3], N. Angerhofer[1], N. M. Myres[1], A. Torroni[2], S. R. Woodward[1]* 1) Sorenson Molec Genealogy Fndn, Salt Lake City, UT; 2) Dip. di Genetica e Microbiologia, Università di Pavia, Pavia, Italy; 3) Dip. di Biologia Cellulare e Ambientale, Università di Perugia, Perugia, Italy.

Haplotypes derived from the maternally-inherited mitochondrial DNA (mtDNA) control region are often employed as a first step in determining phylogenetic-relevant samples that could be selected for additional coding region testing. Using the currently defined world mtDNA haplogroup tree, researchers can assign these haplotypes to specific branches, paying particular attention to novel mutations that could assist in identifying new subclades. During a recent survey of the nearly 58000 mtDNA control region haplotypes currently present in the publicly accessible Sorenson Molecular Genealogy Foundation database, we observed a small number of mtDNAs (n=16) characterized by the presence of unusually long insertions of up to 200 bases. A small subset of these particularly long mtDNA haplotypes shared an identical insertion of 15 bases. Genealogical analysis combined with haplogroup prediction confirmed that these haplotypes shared a common African origin. Additionally, based on the pedigree data gathered, we determine the donors were not closely related. Moreover, through the analysis of complete mtDNA sequences, we conclude that the newly defined haplogroup is most likely of recent origin. As reported in this study, insertions of more than 10 bps are quite rare in the general population and in the published literature, thus providing an interesting case work in population and possibly future disease studies.

Comperhensive approach to autosomal recessive non-syndromic hearing loss in Iranian Population. *H. Najmabadi[1], C. Nishimura[2], N. Meyer[2], T. Yang[2], K. Kahrizi[1], N. Bazazzadegan[1], M. Mohseni[1], Y. Riazalhoseini[1], G. Asaadi Tehrani[1], A. Daneshi[3], M. Farhadi[3], S. Yahyavi[3], E. Taherzadeh[1], P. Imani[1], A. Anousheh[1], A. Nazeri[1], S. Abedini[1], N. Nikzat[1], S. Arzhangi[1], R. J. H. Smith[2]* 1) Gen Res Ctr, Univ Soc welfare & Rehab Sc, Tehran, Iran; 2) Molecular Otolaryngology Research Laboratories, Department of Otolaryngology, University of Iowa, Iowa, IA, United States; 3) Research Center of Ear, Nose, Throat, and Head and Neck Surgery, Iran university of Medical sciences, Tehran, Iran.

Genetic testing for deafness in Iran is well established. The population is extremely heterogeneous, which means that ethnic-specific data are required. We have generated much of these data by screening over 2000 families segregating autosomal recessive non-syndromic deafness (ARNSD). All patients were screened for mutations in GJB2 and GJB6 (DFNB1), and if no mutations were identified, haplotypes were reconstructed by typing three short tandem repeat polymorphisms flanking 22 known ARNSD loci. In a subset of families, genome-wide linkage analysis was completed. Our data show that 16.7% of the Iranian population with ARNSHL segregates mutations in GJB2. The most prevalent mutation in this gene was 35delG, although more than 20 mutations have been identified, five of which are unique to the Iranian population. We also identified novel GJB2 mutation in an endogenous population segregating ADNSHL in village north of Iran. For approximately 30% of families, we have been able to establish a genetic cause for deafness. Over half have mutations in GJB2, and after GJB2, mutations in SLC26A4 and TECTA are most commonly detected. We have also found mutations in PJVK , TMC1 and USH1C, OTOF, MYOVIIA, VLGR1 and genes in our families. In addition, we have described a new syndrome, a contiguous gene deletion syndrome that involves both deafness and infertility in males. The data from the Iranian population attest to its diversity and contribute to the current body of knowledge regarding the deafness of genetics.

Genetic analysis of Iranian families with usher syndrome type 1, 2, 3. *K. Kahrizi[1], G. Asaadi Tehrani[2], S. Saketkhoo[2], N. Bazzazadegan[1], M. Mohseni[1], S. Arzhangi[1], R. J. H. Smith[3], H. Najmabadi[1]* 1) Genetics Research Ctr, GRC, USWR, Tehran, Iran; 2) Genetic Department, Science and Research Branch , Islamic Azad University; 3) Molecular Otolaryngology Research Laboratories , Department of Otolaryngology Head and Neck Surgery , University of Lowa , IA , United States.

Usher syndrome (USH) is an autosomal recessive hereditary disorder characterized by the association of sensorineural hearing loss, retinitis pigmentosa and vestibular dysfunction. It is clinically and genetically heterogeneous and at least 13 chromosomal loci are assigned to three clinical USH typed, namely USH1A-H, USH2A-H and USH3A. There are five known USH1 molecules, in addition, three USH2 genes and one USH3A gene have been identified. Worldwide USH1 and USH2 account for most of the usher syndrome cases with rare occurrence of USH3. In the present study, we performed linkage analysis using STR polymorphic markers for 33 consanguineous Iranian families to examine , all identified loci for USH1 (USH1A, USH1B, USH1C,USH1D,USH1E,USH1F,USH1G and USH1H) , all loci for USH2 (USH2A, USH2B, USH2C,USH2D) and USH3A . Our results showed that ten families linked to one of the loci related to USH1 or USH2: three families linked to DFNB12, two families linked to DFNB2, one family linked to DFNB23 and one family showed linkage for USH1C, also linkage analysis for USH2 loci showed that one family linked to USH2A, and two families linked to USH2C, in which concludes USH1D, USH1B, USH1F, USH1C, USH2A and USH2C. Mutation analysis for MYO7A gene in one family showed R150X: 444 C>T in all affected , also one deletion mutation identified in VLGR1b gene , furthermore mutation in exon 5, 463C>T, Arg155X for USH1C detected , and sequencing mutation analysis for other CDH23, PCDH15 and USH2A genes is performing. The present study suggests that mutations in CDH23 , MYO7A , causing non syndromic autosomal recessive deafness DFNB12 , DFNB2 and USH1D, are the two major components of causes for USH1, while USH1C, and SANS are less frequent causes, also VLGR1b should have more prevalent from other USH2 genes .

Carrier Frequency of SMA by Quantitative Analysis of the SMN1 deletion in the Iranian Population. *M. Hasanzad[1,2], M. Azad[3], K. Kahrizi[1], S. H. Tonekaboni[4], S. Nafisi[5], Z. Keyhanidoust[6], B. Shoja Saffar[1], A. Aghajani[1], S. H. Jamaldini[1], H. Najmabadi[1,3]* 1) Genetics Research Center, University of Social , Tehran, Tehran, Iran; 2) Islamic Azad University, Tehran Medical Branch, Tehran, Iran; 3) Kariminejad-Najmabadi Pathology & Genetics Center, Tehran, Iran; 4) Mofid Hospital,Tehran,Iran; 5) Shariati Hospital,Tehran, Iran; 6) Imam Khomeini Hospital, Tehran, Iran.

Spinal muscular atrophy (SMA) is a common neuromuscular disorder. The survival motor neuron (SMN) protein is encoded by two genes, the telomeric and centromeric SMN. This disease is caused by mutation in the telomeric copy of the SMN gene. Carrier frequency studies of SMA have been reported for various populations. Although no large-scale population-based studies of SMA have been performed in Iran, previous estimates have indicated that the incidence is much higher in the Iranian population compared to that of other populations, partly because of the high prevalence of consanguineous marriage. In this study, we use a reliable and highly sensitive quantitative real-time PCR assay with SYBR green I dye to detect the copy number of the SMN1 gene to determine the carrier frequency of SMA in 200 Iranians. The homozygous SMN1 deletion ratio among the patients was 0.00 and the hemizygous SMN1 deletion ratio among the parents of the patients ranged from 0.29 to 0.55. The Ct ratios of 46 persons among 200 normal individuals were within the carrier range of 0.31-0.57, revealing a carrier frequency of 20% in the Iranian population. Our data confirms that the SMA carrier frequency is much higher in the Iranian population compared to that reported for many other countries.

De novo copy number variations in children with sudden infant death. *G. A. Toruner[1,2], R. Kurvathi[2], R. Sugalski[2], L. Shulman[2], S. Twersky[2], P. Goldblatt Pearson[2], R. Tozzi[3], M. Schwalb[1,2], R. Wallerstein[2]* 1) The Genetics and Genetics Counseling Program,Dept of Pediatrics at The Joseph M. Sanzari Childrens Hospital,Hackensack University Medical Center, Hacksensack, NJ; 2) Institute of Genomic Medicine, Dept of Pediartics, UMDNJ - NJ Medical Sch, Newark, NJ; 3) Pediatric Center for Heart Disease ,Dept of Pediatrics at The Joseph M. Sanzari Childrens Hospital,Hackensack University Medical Center, Hackensack, NJ.

Background: Sudden death of an infant is a devastating event that needs an explanation. When an explanation cannot be found, the case is labeled as Sudden Infant Death Syndrome (SIDS) or Unclassified Infant Death (USID). The influence of genetic factors has been recognized for sudden infant death, but copy number variations (CNVs) as potential risk factors have not been evaluated yet. Methods: Twenty-seven families were enrolled in this study. The tissue specimens from deceased children were obtained and array based comparative genomic hybridization experiments (array-CGH) were performed on the genomic DNA isolated from these specimens using custom Agilent 4x44K arrays. Q-PCR experiments were performed to confirm the overlapping duplication and deletion region in two different cases. Results: A de-novo CNV is detected in three of twenty-seven cases (11%). In case 1, a ~3 Mb (chr8: 143,211,215- qter) duplication on 8q24.3-qter and a 4.4 Mb deletion on the 22q13.3-qter (chr 22: 45,047,068- qter) were detected. Subtelomeric chromosome analysis of the father and the surviving sibling of case 1 showed a balanced reciprocal translocation, 46,XY t(8;22)(q24.3;q13.3). A 240kb (chr 6: 26,139,810-26,380,787) duplication and a 1.9 Mb deletion (chr 6: 26,085,971-27,966,150) at chromosome 6p22 were found in cases 2 and 3 respectively. Array-CGH and conventional cytogenetic studies did not reveal the observed CNVs in the parents and the siblings of the cases 2 and 3. The detected CNVs in cases 2 and 3 encompassed several genes including the major histone cluster genes. Conclusion: Array-CGH analysis may be beneficial during the investigations after sudden infant death.

Polymorphism in Cyclooxygenases-2 (COX-2 / PTGS-2) and liver cancer in Chinese. *N. Tang[1,2], J. Y. Jiang[1], W. Yeo[3], P. S. Lai[4]* 1) Dept Chemical Pathology, Chinese Univ Hong Kong, Hong Kong, SAR, China; 2) Laboratory of Genetics of Disease Susceptibility, Li Ka Shing Institute of Health Sciences, Prince of Wales hospital, Hong Kong, SAR, China; 3) Dept of Clinical Oncology, The Chinese University of Hong Kong, Hong Kong, SAR, China; 4) Dept of Surgery, The Chinese University of Hong Kong, Hong Kong, SAR, China.

Hepatocellular carcinoma (HCC) is an important cancer in the Southern Chinese population and many are related to chronic HBV hepatitis and cirrhosis. The importance of the prostaglandin pathway in the carcinogenesis of HCC has been implicated by treatment of COX-2 inhibitors in animal models of HCC, where COX-2 inhibitor was protective against development of HCC. COX-2 is the inducible isoenzyme and we previously identified a putative functional SNP in the coding gene for COX-2, PTGS-2. Here we examined the association between this 3-UTR SNP and HCC in a Chinese patient sample. Genomic DNA was extracted from peripheral blood collected from 388 Chinese HBV-related HCC patients and 152 sex-matched controls (HBV carriers). This 3-UTR SNP (rs5275, Ex10+837 T/C) represents one of the 4 tagging SNPs we identified from the Han HapMap data. Genotyping was performed by PCR-RFLP as described in the references. There was a borderline association between rs5275 and HCC(p value of genotypic association=0.07 and allelic association = 0.04). The T allele was more prevalence among HCC patients (0.84 vs 0.78 in control). Previous studies of polymorphisms in PTGS-2 were largely confined to a promoter SNP -765G/C (rs20417), however this SNP was not polymorphic in Chinese with MAF less than 0.05. Therefore, we investigated other potential functional or tagging SNPs of this inducible COX gene. The 3-UTR SNP was a potential functional SNP located within a region which had been shown to influence mRNA stability. Furthermore, it had been associated with risk of lung cancer recently. We are expanding our sample size to further validate this association.

Molecular Mechanism and Characterization of Maternally Inherited Essential Hypertension. *S. W. Wang[1], Z. B. Li[1], Y. Q. Liu[1], H. Y. Zhu[1], Y. S. Zhao[1], C. Y. Lu[1], Y. H. Li[1], Y. Wen[1], M. X. Guan[2, 3]* 1) Geriatric Cardiology, Institute of Geriatric Cardiology, Chinese PLA General Hospital, Beijing, China; 2) Division of Human Genetics, Cincinnati Childrens Hospital Medical Center, 3333 Burnet Avenue, Cincinnati, OH 45229-3039, USA; 3) Deparment of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, OH, USA.

Objective:We aimed to observe the relationship between the mitochondrial tRNA mutation and the essential hypertension by examining the mutation of tRNA. We also wanted to explore the inherited signs and clinical characters of maternally inherited essential hypertension. Methods: We collected the clinical data subjects. We extracted DNA from subjects white blood cell, and amplificated the target fragment using the special primers. We then purified the PCR products, and then we directly sequenced them. We also made a comparative analysis of the collected data of the essential hypertension subjects who carried tRNA mutation and those who did not. Results: From the mutation analysis of mitochondrial DNA of 2,000 essential hypertensive subjects, we totally found 26 mutation sites in 57 subjects, in which 22 mutation sites were new. The most frequently occurrence of the mutation site was A4386G in tRNAGln gene. The onset ages of the individuals carrying the mutation were earlier than those who did not bear them. tRNA mutations significantly affected serum lipids, blood electrolyte, blood creatinine, blood urea nitrogen and heart structure and function. Most essential hypertensive patients had maternally inherited history, which fulfilled the feature of mitochondrial hereditary. Conclusion: Mitochondrial tRNA mutations might result in the change of their structure and function, and then damaged the blood metabolism, the balance of the blood electrolyte, the steady-state of the blood cells and the heart structure and function, which were involved in the progress of the essential hypertension. Part of the essential hypertension patients clinically presented the characters of maternal inheritance, which might be associated with the tRNA mutation.

Maternally inherited hypertension is associated with the mitochondrial tRNAIle A4295G mutation in a Chinese family. *Z. B. Li[1], S. W. Wang[1], Y. Q. Liu[1], L. Yang[2], M. X. Guan[2,3]* 1) Institute of Geriatric Cardiology, Chinese PLA General Hospital, Beijing, China; 2) Division of Human Genetics, Cincinnati Childrens Hospital Medical Center; 3) Deparment of Pediatrics, University of Cincinnati College of Medicine.

Objective: Mutations in mitochondrial DNA have been associated with cardiovascular disease. We report here the clinical, genetic, and molecular characterization of one three-generation Han Chinese family with maternally transmitted hypertension. Methods: Members of this Chinese family underwent a physical examination, laboratory assessment of cardiovascular disease risk factors, and routine electrocardiography. Genomic DNA was isolated from whole blood and the entire mitochondrial gene was amplified by PCR. PCR fragments were purified and subsequently analyzed by direct sequencing analysis. Results: Sequence analysis of the complete mitochondrial DNA in this pedigree revealed the presence of the known hypertension-associated tRNAIle A4295G mutation and 33 other variants, belonging to the Asian haplogroup D4j. The A4295G mutation, which is extraordinarily conserved from bacteria to human mitochondria, is located at immediately 30 end to the anticodon, corresponding to conventional position 37 of tRNAIle. The occurrence of the A4295G mutation in several genetically unrelated pedigrees affected by cardiovascular disease but the absence of 242 Chinese controls strongly indicates that this mutation is involved in the pathogenesis of cardiovascular disease. Of other variants, the tRNAGlu A14693G and ND1 G11696A mutations were implicated to be associated with other mitochondrial disorders. The A14693G mutation, which is a highly conserved nucleoside at the TwC-loop of tRNAGlu, has been implicated to be important for tRNA structure and function. Furthermore, the ND4 G11696A mutation was associated with Leber s hereditary optic neuropathy. Conclusion: The combination of the A4295G mutation in the tRNAIle gene with the ND4 G11696A mutation and tRNAGlu A14693G mutation may contribute to the high penetrance of hypertension in this Chinese family.

Effect of voltage-dependent anion channel on the apoptosis of cell lines carrying mitochondrial DNA A4263G mutation. *S. Wang[1], Y. Q. Liu[1], Z. B. Li[1], Y. li[1], H. Xu[2], Y. Wen[1], L. Wang[1], R. Chen[1], M. H. Liu[1], M. X. Guan[3]* 1) Institute of Geriatric Cardiology, Chinese PLA General Hospital, Beijing, China; 2) Military Medical Science Academy of the Chinese PLA; 3) Cincinnati Childrens Hospital Medical Center, Division of Human Genetics.

Objective: In this report, we studied the effect of voltage-dependent anion channel (VDAC) on the apoptosis of the cell lines carrying mitochondrial DNA A4263G mutation. Methods: We established lymphoblastoid cell lines from 3 symptomatic and 1 aymptomatic hypertension individuals in the family carrying A4263G mutation compared with 3 control cell lines. The mitochondrial potential was detected by flow cytometry and the co-localization of VDAC and Bax was evaluated by confocal microscopy. Results: The results showed that the expression of VDAC and Bax in individuals carrying mtDNA A4263G mutation increased compared with control group, while the expression of small conductance calcium dependant potassium (sKCa) had no change. Flow cytometry showed mitochondrial potential (m) of cell lines from individuals carrying mtDNA A4263G mutation decreased 32% compared with control group (P0.05) and this difference was attenuated by Cyclosporin A (CsA), which was a blocker of VDAC. The confocal scanning showed co-localization of VDAC/Bax on the membrane of mitochondrial in individuals carrying mtDNA A4263G mutation, while the combination was not seen on control group. Conclusion: In conclusion, the change of expression of mitochondrial VDAC and subcellular localization of VDAC/Bax leads to the significant increase of mitochondrial permeability and apoptosis of cell lines carrying mtDNA A4263G mutations.

P-values may not provide optimal ranks of true associations in whole-genome scans. *D. V. Zaykin[1], L. A. Zhivotovsky[2], W. Czika[3], S. Shao[3], R. D. Wolfinger[3]* 1) Dept Biostatistics, NIEHS/NIH, Res Triangle Park, NC; 2) Vavilov Institute of General Genetics, Moscow, Russia; 3) SAS Institute, Inc., NC.

In the context of a large collection of statistical genetics tests in which the number of true associations (TAs) is small, we study the distribution of the ranks of TAs among the false associations (FAs). We investigate the relative efficiency of ranking measures and how many "best" results need to be screened to cover TAs with high probability, using a few different ways of assessing significance and adjusting for multiple testing. This way of looking at the problem can aid in optimally following up on initial significant findings and in planning of future large scale experiments. Genome-wide expression studies and whole-genome association scans are prominent examples where the number of tests can range from tens to hundreds of thousands. The measure of association with a trait of interest could be a p-value, possibly weighted towards the effect size. Under a wide set of conditions, we study rank distribution of the TA p-values amongst a large number of FAs, while taking into account the impact of multiple testing adjustments. We demonstrate theoretically, and confirm by simulations, that there are situations where sorting results by the effect size produces better ranks of TAs than the usual sorting by a test statistic value, or by a p-value.

Replication of candidate genes, locus-wide association study for IQ. *T. S. Rizzi[1], A. Arias-Vásquez[2], B. Neale[3], J. Lasky-Su[12], R. Anney[4], P. Asherson[3], E. Sonuga-Barke[5], M. Gill[4], J. Sergeant[13], R. Ebstein[6], A. Rothenberger[7], H. C. Steinhausen[8], T. Banaschewski[7], R. Oades[9], A. Miranda[10], H. Roeyers[11], J. Buitelaar[2], D. Posthuma[1], B. Franke[2], S. V. Faraone[14]* 1) Department of Biological Psychology, Vrije Universiteit, Amsterdam, Netherlands; 2) Department of Psychiatry, Radboud University, Nijmegen Medical Center, Nijmegen, The Netherlands; 3) MRC Social Genetic Developmental and Psychiatry Centre, Institute of Psychiatry, London, UK; 4) Department of Psychiatry, Trinity Centre for Health Sciences, St James's Hospital, Dublin, Ireland; 5) School of Psychology, University of Southampton, Highfield, Southampton, UK; 6) S Herzog Memorial Hospital, Jerusalem, Israel; 7) Child and Adolescent Psychiatry, University of Göttingen, Göttingen, Germany; 8) Department of Child and Adolescent Psychiatry, University of Zurich, Zurich, Switzerland; 9) University Clinic for Child and Adolescent Psychiatry, Essen, Germany; 10) Department of Developmental and Educational Psychology, University of Valencia, Valencia, Spain; 11) Departments of Experimental Clinical Health Psychology, Ghent University, Ghent, Belgium; 12) Department of Psychiatry, Harvard Medical School; 13) Vrije Universiteit, De Boelelaan, Amsterdam, Holland; 14) Departments of Psychiatry and Neuroscience and Physiology, SUNY Upstate Medical University, Syracuse, NY, USA.

During the past decades, a handful of candidate gene association and genome-wide studies of specific and general cognitive abilities have been reported for human intelligence. We therefore decided to conduct a replication effort of all currently reported genomic regions of interest for general intelligence. Participants were 947 families from the International Multi-center ADHD Genetics(IMAGE) project. 13.290 SNPs within previously reported candidate genes and genomic regions for human intelligence were analyzed. Six associations yielded $p10^{-5}$ and are located in chromosome 6. Most of the candidate genes did not present significant p-values with the exception of DTNBP1 and APOE ($p10^{-3}$). The function of the most significant genes is not completely clear, yet, and additional studies are needed to validate these results.

Genetic variations of CYP2C9 in 724 Japanese individuals and their impacts on the antihypertensive effects of losartan. *T. Yin[1 2], K. Maekawa[3], K. Kamide[4], Y. Saito[3], H. Hanada[2], K. Miyashita[5], Y. Kokubo[6], Y. Akaiwa[5], R. Otsubo[5], K. Nagatsuka[5], T. Otsuki[5], T. Horio[4], S. Takiuchi[4], Y. Kawano[4], K. Minematsu[5], H. Naritomi[5], H. Tomoike[6], J. Sawada[3], T. Miyata[2]* 1) Institute of Geriatric Cardiology, General Hospital of People's Liberation Army, Beijing, China; 2) Research Institute, National Cardiovascular Center, Osaka, Japan; 3) Division of Functional Biochemistry and Genomics, National Institute of Health Sciences, Tokyo, Japan; 4) Division of Hypertension and Nephrology, Department of Medicine, National Cardiovascular Center, Osaka, Japan; 5) Cerebrovascular Division, Department of Medicine, National Cardiovascular Center, Osaka, Japan; 6) Department of Preventive Cardiology, National Cardiovascular Center, Osaka, Japan.

CYP2C9, a drug-metabolizing enzyme, converts losartan, an angiotensin II receptor blocker, to its active form for the antihypertensive effect. We resequenced CYP2C9 in 724 Japanese individuals including 39 hypertensive patients under the treatment of losartan. Of two novel missense mutations identified, the Arg132Gln variant showed a five-fold lower intrinsic clearance toward diclofenac when expressed in a baculovirus-insect cell system, while the Arg335Gln variant had no substantial effect. Several known missense variations were also found, and approximately 7% of the Japanese individuals (53 out of 724) carried either one of the deleterious alleles (CYP2C9*3, *13, *14, *30, and Arg132Gln) as heterozygotes. After 3 months of losartan treatment, the systolic blood pressure was not lowered in two patients with CYP2C9*1/*30, suggesting their impaired in vivo CYP2C9 activity. CYP2C9*30 might be associated with the diminished response to antihypertensive effects of losartan.

New Mutations Identified in Hermansky Pudlak Syndrome, Subtype 3 and Case Reports of Two Children With This Subtype. *G. A. Golas, R. Hess, A. Helip-Wooley, M. Huizing, R. Fischer, K. O'Brien, T. Markello, W. Westbroek, W. A. Gahl* Medical Genetics Branch NHGRI/NIH 10 Center Drive Bethesda, Md 20892.

Hermansky Pudlak Syndrome (HPS) is an autosomal recessive disdorder exhibiting both genetic and clinical heterogeneity with 8 distinct subtypes identified at specific loci, and characterized by oculocutaneous albinism, a bleeding diathesis, and variably, in some subtypes, pulmonary fibrosis and granulomatous colitis. We have diagnosed and described the clinical and molecular findings of 29 patients with HPS 3 in a cohort of 232 individuals with HPS studied in the NIH protocol 95-HG-0093. Analysis of gene map locus 3q24 is reported here and revealed 19 different mutations: 5 with an Ashkenazi Jewish mutation in the homozygous state; 9 with the central Puerto Rican HPS mutation in a homozygous state; and 15 others carrying 17 varied mutations, 12 of which are novel and unpublished. Two unrelated children, ages 7 and 10, diagnosed with HPS 3, confirm the previously-documented milder clinical phenotype of HPS 3, reflecting near normal skin/hair hypopigmentation, typical ocular findings, and only a very mild bleeding tendency. Neither had any significant gastrointestinal involvement and both are too young to exhibit the pulmonary fibrosis which characterizes subtypes 1 and 4, not HPS 3. Patient 1, of consanguineous French Canadian parents, has blonde hair, blue eyes, pale skin, visual acuity 20/160-250, nystagmus, photophobia, easy bruisability and a history of one minor nosebleed. A chin laceration requiring sutures had no prolonged bleeding. Patient 2, of nonconsanguineous Jewish and Irish/French/German parents, has brown eyes, brown hair, olive skin, nystagmus, visual acuity of 20/60-80, easy bruisability, and a history of two episodes of epistaxis controlled with intranasal DDAVP. Identification of a milder subtype of HPS without the prognosis of fatal pulmonary fibrosis in early adulthood can give enormous relief to parents of these children and guide management of their milder bleeding manifestations.

Histone Deacetylase Inhibitors Differ in Regulating Cardiac Dysfunction in Response to Pressure Overloaded. *J. Ma[1,2], T. Minamino[2], Y. Asano[2], Y. Liao[2], M. Kitakaze[3], S. Wang[1]* 1) Institute of Geriatric Cardiology, Chinese People's Liberation Army General Hospital, Beijing, China; 2) Department of Cardiovascular Medicine , Osaka University Graduate School of Medicine, Osaka, Japan; 3) Department of Cardiovascular Medicine , National Cardiovascular Center, Osaka, Japan.

Recent work has uncovered that histone acetylation might work as a nodal point in the signal network for gene regulation in hypertrophic myocardium, moreover, histone deacetylase(HDAC) inhibitors might hold promise as potential therapeutic agents in hypertrophic heart disease. However differential class effects of HDAC inhibitors on cardiac dysfunction in response to pressure overload have not been fully elucidated. In this study, we tested the effects of 4 types of HDAC inhibitors, trichostatin A (TSA) ,valproic acid (VPA), 4-Sodium phenylbutyrate (PBA), and butyrate acid (BS). All the 4 inhibitors successfully inhibited HDAC total activity both in vivo and in vitro. Individual HDAC activity assay showed rather than class IIa members (HDAC 4 and 7), PBA and BS predominantly inhibit classIHDACs (HDAC2 and 8), whereas VPA and TSA inhibit them all to a similar extent. To investigate their effects on cardiac function, transverse aortic constriction (TAC) operation was performed on C57BL mice. Cardiac hypertrophy and dysfunction induced by TAC for 6 weeks were significantly blunted by administrations of TSA and VPA, as reported previously. In contrast, PBA and BS treatments unexpectedly exaggerated cardiac remodeling and dysfunction indicated by histological and echocardiogram results. Invasive hemodynamic measurements demonstrated higher LV end diastolic pressure (PBA:31.0, BS: 29.9, vehicle: 15.5 mmHg, $p<0.001$) and lower contractivity index (PBA:120.8, BS: 118.7, vehicle:160.4, $p<0.01$). Kaplan-Meier analysis also revealed significantly lower survival rate (PBA: 32.7%, BS: 30.8%, vehicle: 66.7%, $p<0.001$). Together, these data suggest that PBA and BS accelerate load-induced hypertrophy and cardiac dysfunction and increase mortality, whereas VPA and TSA hold opposing effects. The differential regulation pattern on HDACs might account for this.

JAZF1 is a novel risk gene for type 2 diabetes: the DiaGen consortium. *J. T. Salonen[1], O. Kontkanen[1], P. Uimari[1], M. Pirskanen[1], J. Hyppönen[1], T.-P. Tuomainen[2], J. Luedemann[3], W. Kerner[4], U. Kowalik[5], D. Meyre[6], J. Delplanque[6], P. Froguel[6]* 1) Oy Jurilab Ltd, Kuopio, Finland; 2) University of Kuopio, Kuopio, Finland; 3) Ernst Moritz Arndt University, Greifswald, Germany; 4) Center of Cardiology and Diabetes, Karlsburg, Germany; 5) Grochowski Hospital, Warsaw, Poland; 6) Institut Pasteur de Lille, Lille, France.

Progress in the development of genotyping technologies has resulted in a burst of genome wide association (GWA) studies identifying multiple new genetic loci associated with type 2 diabetes (T2D). Subsequent meta-analysis of the three GWAs followed by a large replication has brought our attention to at least six new gene regions having a modest effect on susceptibility to T2D. Following our GWA study DiaGen, we carried out a replication study of almost 1400 single nucleotide polymorphisms (SNPs) in 2706 individuals from France, Germany and Poland. The strongest evidence for association was detected in the previously identified TCF7L2, CDKAL1 and EXT2 loci, and in the CDKN2A - CDKN2B and HHEX - IDE regions. SNPs flanking the JAZF1 gene, the strongest new locus in the recent meta-analysis, showed a significant association with T2D (P = 2.24×10-8) after Bonferroni correction. Our present study provides further support for an association of the JAZF1 intron 1 region with T2D, and further elaborates the association of the JAZF1 upstream regulatory region with T2D. Our present results, together with the meta-analysis by Zeggini and co-workers suggest that JAZF1 is a gene that contributes to the predisposition and most likely to the etiology of T2D.

Replication study for the Wellcome Trust Case Control Consortium data in large-scaled Japanese rheumatoid arthritis case-control populations. *S. Tsukahara[1], K. Ikari[1], Y. Kochi[2], K. Yamamoto[2], N. Yasui[3], H. Inoue[4], M. Itakura[4], M. Hara[1], H. Yamanaka[1], N. Kamatani[1], S. Momohara[1]* 1) Inst of Rheumatology, Tokyo Women's Med Univ, Tokyo, Japan; 2) Dept of Allergy and Rheumatology, Graduate School of Medicine, the University of Tokyo, Tokyo, Japan; 3) Dept of Orthopedics, Inst of Health Biosciences, The University of Tokushima Graduate School, Tokushima, Japan; 4) Inst for Genome Research, The University of Tokushima, Tokushima, Japan.

INTRODUCTION. Rheumatoid arthritis (RA) is believed to be a complex disease that is influenced by both genetic and environmental factors. Recently, the Wellcome Trust Case Control Consortium (WTCCC) published the result of GWAS in a British population. In addition to SNPs within HLA region and PTPN22, nine SNPs were found to be associated with RA. Moreover, another SNP at 6q23, rs10499194, was reported to be associated with RA using GWAS in U.S., which is located 3.8kb from rs6920220, one of the nine associated SNPs. To replicate these findings in Japanese, we performed an association study using three independent cohort.

METHODS. Genotyping was performed using the TaqMan method. IORRA cohort, which included 1504 cases with 752 controls, was used for the first stage of the association study. When an association was found, two other cohort were used to evaluate the result; RIKEN/BioBank (1113 cases and 940 controls) and Tokushima (950 cases with 507 controls). Associations of the SNPs with RA were estimated by Chi-squared test and the results among independent cohort were combined with the use of the Mantel-Haenszel method.

RESULTS. We could not support the evidence of association between the nine WTCCC SNPs and RA (P= 0.09-0.77, rs6920220 was not polymorphic in a Japanese population). Though we found an association between rs10499194 at 6q23 and RA in the IORRA cohort (P= 0.039), the latter stage of the study revealed no association between them (combined P= 0.08).

CONCLUSION. We conclude that the nine WTCCC SNPs do not have big impact on RA susceptibility in Japanese.